Fall 12-20-2019

The effectiveness of Resin-Modified Glass Ionomer Varnish in Preventing White Spot Lesions During Fixed Appliance Orthodontic Therapy: An In Vitro Study

Bradley Herman
University of Nebraska Medical Center

THE EFFECTIVENESS OF RESIN-MODIFIED GLASS IONOMER VARNISH IN PREVENTING WHITE SPOT LESIONS DURING FIXED APPLIANCE ORTHODONTIC THERAPY: AN IN VITRO STUDY

By

Bradley Michel Herman

A THESIS

Presented to the Faculty of

the University of Nebraska Graduate College

in Partial Fulfillment of Requirements

for the Degree of Master of Science

Medical Sciences Interdepartmental Area
Oral Biology

Under the Supervision of Professor Mark Beatty

University of Nebraska Medical Center
Omaha, Nebraska

December 2019

Advisory Committee:

Sundaralingam Premaraj, B.D.S., M.S., Ph.D., FRCD(C)

Peter J. Giannini, D.D.S., M.S.

Gregory G. Oakley, Ph.D.

ACKNOWLEDGMENTS

I would like to thank Dr. Mark Beatty for all of his help in developing this project and advising me all along the way. Your help was invaluable throughout this whole process and this project could not have been completed in its current form without it. You were always willing to answer my questions and gave me great ideas that made this thesis even better. Thank you.

I would also like to thank the members of my committee: Drs. Premaraj, Giannini, and Oakley. Thank you for all of your help, support, and input. It is truly appreciated.

Thank you to Dr. Joe Zhu and Julia "Jules" Russ at the Morrison Microscopy Core Research Facility for helping me with the scanning electron microscope images of my samples. Thank you for all the time that you spent helping me get the best images possible.

Thank you to Jennifer Rooney with the Canary System for your help in training me to use the Canary System and for answering all of my questions.

I would also like to thank my wife for supporting me throughout this project, and for helping me get everything done on time. I know that I could not have done it without you. I love you.

Finally, and most importantly, I would like to thank God for this opportunity that He has given me. Without His help and guidance, I would not be the person that I am today. Without Him, I can do nothing.

THE EFFECTIVENESS OF RESIN-MODIFIED GLASS IONOMER VARNISH IN PREVENTING WHITE SPOT LESIONS DURING FIXED APPLIANCE ORTHODONTIC THERAPY: AN IN VITRO STUDY

Bradley M. Herman, D.D.S., M.S.

University of Nebraska, 2019

Advisor: Mark Beatty, D.D.S., M.S.E., M.S.D., M.S.

The purpose of this investigation was to evaluate the efficacy of a glass ionomer varnish in preventing demineralization of enamel adjacent to and some distance away from an orthodontic bracket. Forty extracted human molars were divided into four different treatment groups (varnish in distilled water, varnish in acetic acid, enamel in acetic acid, and enamel in distilled water). Two 3 x 3 mm graphite windows were drawn on the tooth, one adjacent to the bracket and one 3 mm away from the first window, indicating the scanning area. Varnish was applied to the teeth in the varnish groups and all the teeth were immersed in their respective solutions. Demineralization level was determined with the use of the Canary System at baseline, 3 days, 7 days, and 14 days. Scanning electron microscope images were obtained at baseline and 14 days. The results of this study suggested that glass ionomer varnish is effective in preventing demineralization of the enamel surface of teeth with orthodontic brackets in patients with a high caries risk. The results of this study also suggested that enamel demineralization occurs independently of the proximity to the bracket margin, and that the Canary System can be a useful instrument in assessing enamel demineralization over time around bracket margins and under resin-modified glass ionomer.

TABLE OF CONTENTS

LIST OF FIGURES

LIST OF TABLES

CHAPTER 1: INTRODUCTION

White spot lesions (WSLs) are defined as “the first sign of a caries lesion on enamel that can be detected with the naked eye” (Fejerskov and Kidd 2003). On the continuum from healthy, intact enamel to frank cavitation, WSLs are considered the first step towards the development of a carious lesion. Without appropriate prevention or treatment of existing WSLs, more serious carious lesions will likely develop. Unfortunately, the development of WSLs on the facial surfaces of the teeth is common during fixed appliance orthodontic therapy. The prevalence of WSLs during orthodontic therapy reported in the literature varies from 15% to 85% (Mitchell 1992), but many studies report a prevalence of 50% or higher (Mizrahi 1983; Sundararaj et al. 2015), with some estimates as high as 75% (Wenderoth et al. 1999). The most likely explanation for the development of WSLs during fixed appliance therapy is that the long-term, fixed nature of the appliance increases the propensity of the tooth surface to collect plaque and debris as well as hindering the patient’s ability to adequately cleanse the tooth surfaces around the appliance components (Geiger et al. 1988). WSL development in these patients may be partially due to the increased levels of the microorganism *Streptococcus mutans*, which has been implicated in caries development (Corbett et al. 1981). WSL development can be relatively rapid, with clinically evident WSLs present in as little as one month in orthodontic patients (O'Reilly and Featherstone 1987). Thus, fixed appliance orthodontic therapy thus, in the presence of inadequate oral hygiene, provides more favorable conditions for the development of WSLs (Gorelick et al. 1982). There exists a need, therefore, for the development of a material or technique that will minimize or eliminate the development of WSLs during orthodontic treatment.

When brackets are adhered to an enamel surface, there is usually a small amount of composite resin that extends beyond the bracket margin, commonly called flash. The surface is irregular at this site, permitting plaque formation and retention, which poses an increased risk for white spot lesion development. Studies have shown that biofilm formation can be dependent upon

the surface energy of the bonding material, when measured as the contact angle between the adhesive and the tooth surface (Ho et al. 2017). When a varnish, sealant, or other dental material is applied to the tooth surface around a bracket, microscopic voids may be created between the dental material and the flash or bracket margin, thereby providing an area through which cariogenic acid and microorganisms may pass. It has been shown that WSL formation occurs close to the orthodontic bracket margin (Kumar Jena et al. 2015).

There have been numerous attempts to develop techniques or materials that could help prevent the development of WSLs during fixed appliance therapy. These include in-office fluoride varnish, high fluoride toothpastes, and chlorhexidine or fluoride mouth rinses. However, these may or may not be routinely prescribed by the treating orthodontist (Derks et al. 2007). Many of these materials and techniques are dependent upon patient compliance, which, in many cases, is not adequate. Fluoride varnish application at each orthodontic adjustment appointment has been shown to be an effective method for preventing WSL occurrence (Stecksen-Blicks et al. 2007). However, it requires increased chair time for each patient and its efficacy can be inhibited by poor oral hygiene between appointments, which can be as long as six to twelve weeks apart.

Fluoride-containing orthodontic cements have been developed in order to prevent enamel demineralization during orthodontic treatment. Glass ionomer cements exhibit continuous fluoride release, which helps prevent demineralization around orthodontic brackets (Nakajo et al. 2009), but has a bond strength that is much lower than traditional resin cements, leading to increased bracket de-bonding during treatment (Voss et al. 1993). A composite resin fluoride-releasing cement has been developed with sufficient bond strength for orthodontic applications, while exhibiting fluoride release. However, the fluoride release drops off precipitously after 24 hours (Basdra et al. 1996).

White spot lesions are not esthetically pleasing, as they display an opaque white appearance that does not blend well with the surrounding healthy tooth structure, and do not disappear once

the braces are removed. Once WSLs appear, treatment focuses on preventing or reversing the further progression until the appliances are removed. When removed, the focus shifts from prevention to eliminating or camouflaging the WSLs to meet the esthetic demands of the patient. Numerous methods have been attempted to treat WSLs following orthodontic treatment; however, their efficacy has not been supported by the literature (Chen et al. 2013). Completely eliminating WSLs may involve costly and invasive restorative procedures, which can add significant cost to the patient. Since WSL treatment is questionable, prevention, with the aid of newer technologies for early caries detection, appears to be the preferred option (Tassery et al. 2013).

At present WSLs are detected via visual or radiographic examination, where the lesions have progressed to a significant level of enamel demineralization (Manton 2013). Improved treatment outcomes would be realized with earlier detection. Recently, a detection device called the Canary System has been marketed for such purpose. The Canary System utilizes laser technology based on photothermal luminescence to detect demineralization present on an enamel surface. A handpiece emits laser pulses of a certain wavelength onto a tooth surface and then the laser is converted by the enamel to either heat or light. The light and heat are then reflected back from the enamel to the handpiece, which contains a sensor that detects the light and heat. Sound enamel reflects heat and light of a different magnitude and wavelength than carious enamel, which is what allows the Canary System to differentiate various levels of enamel demineralization. These differing levels of light and heat reflection create a unique photothermal and luminescence (PTR-LUM) signal for each enamel site, which is then converted into a numerical score (i.e. Canary Number) by the Canary System. The Canary Number indicates the level of demineralization (Jeon et al. 2007). The Canary System has been compared with the accepted standard for caries detection, the International Caries Diagnostic and Assessment System (ICDAS II), to determine its accuracy *in vivo* for smooth surface and occlusal caries. It was found that the Canary System

had a correlation (R^2) of 0.9759 with the ICDAS II for smooth surface carious lesions and a correlation of 0.9267 for occlusal carious lesions, indicating that the Canary System is capable of distinguishing between sound and carious tooth structure (Silvertown et al. 2017a). This modality of caries detection has been shown to have an accuracy of 76% when used to detect carious lesions under opaque dental sealants when compared to a fluorescence caries detection method (i.e. DIAGNOdent), which had a 59% accuracy. This was confirmed by polarized light microscopy (Silvertown et al. 2017b).

Since it is desirable to reduce or eliminate WSLs, ideally the placement of a material with continuous fluoride-release and adequate bond strength would hinder demineralization and preclude dependence upon patient compliance. Successes have been reported by applying a thin layer of varnish or sealant around the periphery of bonded orthodontic brackets. The varnish is expected to provide a mechanical barrier between intraoral acids and tooth surface, as well as provide extended fluoride release. One such product is Vanish XT, (3M, St Paul, MN) a "resin-modified glass ionomer extended-contact varnish" that is applied at the time of initial bracket bonding and is reported to continuously release fluoride up to six months, independent of patient compliance. It also claims to provide a mechanical barrier against oral acid challenge and does not affect bracket bond strength, as it is applied separately from the bracket luting cement. It has the capability to be re-charged with fluoride during exposure to fluoride-containing materials (e.g. fluoride-containing toothpaste) (3M 2008). Since these manufacturer claims are largely unsubstantiated, the aims of this in-vitro study are to (1) evaluate the efficacy of Vanish XT in preventing enamel demineralization near orthodontic brackets, (2) assess efficacy at enamel sites distant to the margins of an orthodontic bracket, and (3) use the Canary System as a means to estimate enamel demineralization around orthodontic brackets and under varnish.

CHAPTER 2: LITERATURE REVIEW

2.1 White Spot Lesion Development and Orthodontic Treatment

The prevalence of WSLs varies greatly, ranging from 2% to 96% depending upon which diagnostic method is used and the study design employed (Heymann and Grauer 2013). Gorelick et al. compared the prevalence of WSLs in orthodontic patients compared to those who had not undergone orthodontic treatment. They found, regardless of whether using the banded or bonded technique, that orthodontic patients exhibited significantly more WSLs than a control population without orthodontic treatment. They also found that WSLs were more likely found on labial surfaces of maxillary incisors and least likely on maxillary posterior teeth. They did not find any WSLs on lingual surfaces of lower incisor teeth that received a fixed wire retainer. They suggested that the location and development of WSLs in orthodontic patients may be related to salivary flow, and that fluoride treatment should be instituted in conjunction with orthodontic treatment.

A cross-sectional study looked at 527 patients prior to and 269 patients following orthodontic treatment to determine if there were any differences in the prevalence of WSLs between groups. They found that the prevalence of WSLs in the patients that had undergone orthodontic treatment was significantly higher (> 80%) than the prevalence of WSLs in the subjects that had not undergone orthodontic treatment (~ 68%). They also found that the maxillary lateral incisors, maxillary and mandibular first molars, and middle/cervical thirds of labial surfaces on mandibular lateral incisors and canines were the teeth and surfaces where the majority of WSLs were found (Mizrahi 1983). A recent meta-analysis reported that based on fourteen studies, the incidence of new WSLs in orthodontic patients was 46.8% and prevalence was 68.4% (Sundararaj et al. 2015).

In addition to a higher WSL incidence being present in orthodontic patients, the rapidity of lesion formation is of concern. In one study, plaque-accumulating orthodontic brackets were placed on first premolars planned for extraction and scanning electron microscopy (SEM) and

visual analysis methods determined when WSLs began to form. Lesions first appeared in as little as four weeks in the absence of fluoride treatment, and an absence of the surface layer of enamel, indicative of demineralization, was observed with SEM (Ogaard et al. 1988). This finding was supported by Tufecki et al. (2011), where it was found that the prevalence of WSLs was 38% six months into orthodontic treatment and 46% at twelve months, whereas the prevalence of WSLs in the control group was 11%. The authors concluded that orthodontic patients were at an increased risk for WSL development, especially during the first six months of treatment. They suggested using fluoride regimens to prevent demineralization.

The development of WSLs is multifactorial and can be attributed to host factors (e.g. diet), modifying factors in the oral cavity (e.g. orthodontic brackets), and cariogenic microorganisms such as *Streptococcus mutans* and *Lactobacillus* (Heymann and Grauer 2013). In 1981, Corbett et al. studied the prevalence of *Streptococcus mutans*, in patients with banded orthodontic appliances. The amount of *S. mutans* present in plaque was analyzed in patients prior to and during orthodontic treatment. The patients in active treatment had a higher proportion of *S. mutans* (25%) out of the total colony forming units (CFU) of streptococcal species than the proportion in the non-banded patients (5%) and this proportion increased to 32% when only the patients undergoing orthodontic treatment with caries were considered. The authors concluded that the increased numbers of *S. mutans* and infected sites may be related to increased numbers of WSLs in treatment populations.

Flash is considered to be excess resin that flows out from underneath a bracket once it is pressed onto tooth surface. If not removed properly, excess composite resin can act as a site for plaque accumulation (Mei et al. 2011). Plaque accumulation on flash from two adhesives was studied by Ho et al. (2017). Traditional composite resin was associated with more *S. mutans* biofilm formation compared with a fluoride-releasing resin, further supporting the notion that *S. mutans* accumulation pays a role in WSL formation in orthodontic patients.

Although cariogenic bacteria can play a significant role in the development of WSLs, the main contributing factor is plaque accumulation (Ogaard et al. 1988). In the absence of pristine oral hygiene, plaque readily accumulates on orthodontic bracket surfaces and becomes a large community of microorganisms, or biofilm. With frequent exposure to fermentable carbohydrates present in the patient's diet, the biofilm becomes acidified, which repeatedly decreases pH. With continued acidification, the microbial profile shifts towards acidogenic bacteria, leading to increased enamel demineralization and WSL development (Lundstrom and Krasse 1987). Prevention of plaque accumulation and retention becomes critical, and when executed properly and consistently, has been shown to be effective (O'Reilly 1987).

2.2 Prevention of White Spot Lesions

2.2.1 Fluoride-Releasing Cements

One method that has been studied for preventing the development of WSLs is the incorporation of fluoride into the adhesive or cement that bonds the orthodontic bracket to the tooth. The rationale is that the fluoride will be released from the cement during orthodontic treatment, preventing WSLs from forming. One *in vivo* study placed a fluoride-containing orthodontic adhesive on premolars that were planned to be extracted and compared it to a control group, which received an adhesive without fluoride. Using quantitative microradiography to determine the enamel mineral content in volume percent, the authors concluded that the fluoride-containing adhesive was more efficacious at preventing WSLs than the control adhesive (Ogaard et al. 1992). A similar study compared glass ionomer cement with traditional composite resin cement and reported that glass ionomer cement produced significantly fewer WSLs. (Gorton and Featherstone 2003).

Contradictory results were reported in a 1999 *in vivo* study of the maxillary anterior segment in 40 orthodontic patients. In this study, of the 243 teeth studied, half of the teeth in the maxillary anterior segment received a glass ionomer cement during bracket bonding and the remaining teeth

were bonded with composite resin. Clinical photographs and a modified Developmental Defects of Enamel (DDE) Index quantified the level of tooth demineralization. The authors concluded that similar levels of decalcification resulted for brackets bonded with glass ionomer and composite resin, and that oral hygiene and dietary factors were more important contributors to the development of WSLs during orthodontic treatment. The authors discussed that their results may have been different than the results of other similar studies due to the lack of adequate water fluoridation for the subject populations in other studies (Millett et al. 1999).

2.2.2 Orthodontic Appliances

Orthodontic appliances have been implicated in the development of WSLs because tooth cleaning procedures become more challenging and plaque retention/accumulation increases (Geiger et al. 1988). However, the type of bracket material also appears to affect plaque retention and biofilm formation. One *in vitro* study compared biofilm formation on ceramic, plastic, and stainless-steel brackets. Using radioactive labelling, they found a significantly higher adherence percentage on ceramic and plastic brackets (0.55 and 0.44) after 72 hours of *S. mutans* inoculation and placement in a scintillation vial than stainless steel brackets (0.28). The authors mentioned that the surface characteristics of the different bracket materials may have played a role in the different adherence percentages over time (Fournier et al. 1998).

In addition to bracket material differences, bracket design differences have potential for plaque accumulation and WSL development. Certain brackets require manual placement of a ligature to secure an archwire, whereas other brackets have a built-in ligature system. By not requiring a separate ligature, these so-called self-ligating brackets decrease the number of potential areas where plaque can accumulate, which makes tooth cleansing easier. In a study conducted by Pellegrini et al. (2009) using adenosine triphosphate (ATP) bioluminescence, self-ligating brackets accumulated a lower bacterial load than did traditional non-self-ligated brackets. However, there have been other studies that have stated that the method of bracket ligation does

not have an effect on WSL development and that patient oral hygiene and diet modification is of much higher importance. One *in vivo* study separated 20 orthodontic patients into two groups, assigning one group to have self-ligating brackets bonded and the other group to have traditional open-faced brackets bonded. The number of WSLs was counted at the conclusion of treatment, and it was noted that the number of WSLs was the same between groups. The open-faced brackets were ligated with elastomeric ligatures for three months and then with stainless steel ligatures for three months. The authors suggested that diet and environmental factors play a larger role in WSL development than bracket ligation type (Polat et al. 2008). For non-self-ligating brackets, the type of ligature may be important, as elastomeric ring ligatures can harbor more cariogenic bacteria than stainless steel ligatures (Turkkahraman et al. 2005).

2.2.3 Varnishes and Sealants

In 2016, a randomized controlled trial was performed to assess the efficacy of a sealant to prevent WSLs in 50 orthodontic patients. Half of the patients had an organo-selenium sealant coating applied to the facial surfaces of their teeth close to the gingival margin and the other half did not. Oral hygiene instructions were the same for both treatment groups and a plaque index was used to assess oral hygiene at two-month intervals over a 12-month observation period. Poor oral hygiene was determined to be the best predictor for WSL development, with the presence or absence of the sealant appearing to have no effect (Hammad and Knosel 2016).

In another study, a lightly filled Bis-GMA fluoride-releasing sealant was applied to every other anterior tooth and evaluated over a period of five to 18 months. The teeth were evaluated with a visual scale by seven blinded examiners. The sealant did not significantly differ from the control in its ability to prevent the formation of WSLs (Wenderoth et al. 1999).

Other studies have found that sealants can be effective in preventing WSLs during orthodontic therapy. In one study, the authors investigated the ability of a fluoride-releasing varnish to prevent enamel demineralization adjacent to the material. They compared the varnish

to a glass ionomer sealant material and a traditional composite sealant by placing small cavity preparations into the buccal surfaces of extracted teeth and then filling the preparations with one of the materials. They coated the tooth surface up to one millimeter away with an acid-resistant varnish to create an area adjacent to the material for demineralization to occur. The teeth were subjected to an artificial caries environment and then sectioned and analyzed under a polarized light microscope. The fluoride-releasing sealant was able to prevent WSL formation better than the conventional sealant, but not as well as the glass ionomer sealant (Salar et al. 2007).

The efficacy of a resin-modified glass ionomer (RMGI) varnish in preventing WSLs in orthodontic patients was evaluated by Kumar Jena *et al*. (2015). Using a split-mouth design, right or left incisors and canines randomly received a varnish application or no treatment. Both a visual WSL score and laser fluorescence (DIAGNOdent, KaVo dental, Brea, CA) were used to evaluate the varnish at baseline and after six months. The results from both the visual assessment and the DIAGNOdent demonstrated that the RMGI varnish was more effective at preventing WSL development than the control, particularly for maxillary lateral incisors and maxillary and mandibular canines. However, the authors cautioned that the DIAGNOdent scores should be interpreted with caution because although they did show statistically significant differences, that did not necessarily indicate clinically significant or visual differences (Kumar Jena et al. 2015).

Certain studies have investigated fluoride release of varnishes over time. With sustained fluoride release, WSL formation should be inhibited over a longer period of time. In one study, four different varnishes were applied to extracted teeth, submerged in saliva, and fluoride concentration measured after 30 minutes, daily for the first week, and weekly until no more fluoride was detected. It was found that both cumulative and fluoride release over time differed between the varnishes, most likely due to compositional differences. Traditional sodium fluoride varnish released a large amount of fluoride in the first week, but tapered off quickly, while a

resin-modified glass ionomer varnish released less fluoride immediately, but sustained lower levels of fluoride release over an extended time period. (Jablonowski et al. 2012).

Premaraj *et al.* (2017) evaluated two commercially available sealants for fluoride release, bacterial adherence and resistance to acid penetration. Disks were created for each sealant and immersed in double-distilled water. Fluoride release was measured daily for 14 days, and again at 21 and 28 days. To test bacterial adherence, the disks were inoculated with *S. mutans* and *Lactobacillus,* and assessed for the bacterial growth at 24, 48, and 72 hours using propidium iodine staining and confocal microscopy. For acid penetration, varnish was placed on buccal surfaces of extracted teeth, the teeth immersed in 0.1 M lactic acid for four weeks, and microhardness assessed using the Knoop Hardness Test. Acid penetration was assessed by comparing hardness values measured on the coated buccal surfaces versus the corresponding uncoated lingual surfaces, and with scanning electron microscopy. The authors reported that both sealants released fluoride, with significantly higher amounts released by the sealant containing less total percentage of nanofillers. Interestingly, *S. mutans* adherence was greater on the sealant that released more fluoride and *Lactobacillus* adherence was similar for both sealants. Both sealants produced microhardness values that were significantly different between exposed and non-exposed tooth surfaces, indicating effective resistance to acid penetration (Premaraj et al. 2017).

Certain varnishes are intended to be applied once, with coverage expected to be maintained over time, while others are designed to be applied more frequently. Sometimes orthodontists apply the shorter-term varnishes at the beginning of treatment in an effort to prevent the WSL formation. In one study, two varnishes were analyzed for their efficacy in preventing WSLs and gingivitis when applied at the beginning of orthodontic treatment. The investigators visually inspected the tooth surfaces of 90 orthodontic patients using the visual International Caries Detection and Assessment System (ICDAS) and scored the appearance of the WSLs at 4, 12, and

20 weeks post varnish application. It was found that a one-time application of either varnish was not effective in preventing WSL development when compared with the control patients who received a placebo varnish (Kirschneck et al. 2016).

Of the limited number of published research on Vanish XT, a 2018 study compared Vanish XT to another popular sealant to assess differences in WSL prevention. The sealants were applied to extracted human mandibular and maxillary canines and submersed in 0.1 M lactic acid for 32 days. Once the samples were removed from acid, the tooth surfaces were photographed, and both color change and WSL surface area were quantified using computer software. Vanish XT was found to be as effective as the other sealant in preventing WSL formation, and both of the sealants were more effective than the untreated control group (Wiewiora et al. 2018).

2.3 The Canary System and Caries Detection

The diagnosis of carious lesions, particularly non-cavitated lesions, can be a challenge to the dental practitioner, but is essential in preventing caries progression. One method used to diagnose WSLs is visual examination. A number is assigned to the tooth based on its visual appearance to quantify the extent of cavitation. Gorelick et al. 1982 employed a numerical scale from 1 to 4 to indicate the severity of WSLs in orthodontic patients. A score of "1" indicated no WSLs were present and a score of "4" indicated the presence of a cavitated lesion (Gorelick et al. 1982). Other visual inspection methods include the International Caries Detection and Assessment System (ICDAS), which assigns a numerical code to a tooth based on surface and severity of carious lesion. In a systematic review, the ICDAS was shown to have good reproducibility and accuracy for assessing primary coronal lesions (Ekstrand et al. 2018).

Laser fluorescence is also used to detect carious lesions. Both quantitative light-induced fluorescence (QLF) and DIAGNOdent (KaVo Dental, Lake Zurich, IL) have shown some potential for caries detection, but are not equally effective on all tooth surfaces (Zandona and Zero 2006). One *in vitro* study assessed the sensitivity and specificity of DIAGNOdent in

detecting caries around the margins of glass ionomer restorations and found the sensitivity to be 69.8% and the specificity to be 14.3% when measuring directly at the restoration margin (Abrams et al. 2018). Another study compared the accuracy of QLF and DIAGNOdent in assessing *in vitro* smooth surface carious lesions and found that both methods were equally as accurate, but QLF better correlates with enamel mineral content (Shi et al. 2001).

An early caries detection device that was introduced in 2011 is the Canary System (Quantum Dental Technologies, Toronto, Ontario, Canada) (figure 2.1). It uses Photothermal-Luminescence (PTR-LUM) to detect carious lesions prior to their progression to cavitation. The goal of such technology is to diagnose a carious lesion early so that appropriate intervention can be implemented before permanent enamel damage ensues. PTR-LUM directs a laser to the enamel of a tooth, creating a unique photothermal (temperature) and luminescence (light) signature of that area on the tooth. The PTR-LUM signature is analyzed and a numerical score called a Canary Number is assigned (figure 2.2). The number estimates the level of enamel demineralization. A Canary Number of 0-20 indicates healthy tooth structure, 21-70 indicates the presence of decay, and 71-100 indicates the presence of serious decay. The Canary Numbers for each tooth can be stored in a patient's record in order to track changes in Canary Numbers over time (https://thecanarysystem.com/about.php).

One study looked at the correlation between carious lesion depth and the DIAGNOdent, Canary System and ICDAS visual examination scores. Caries in extracted teeth were confirmed histologically with polarized light microscopy. Of the three measurement systems, the Canary System displayed a statistically significant higher correlation with lesion depth than the other two modalities, and an intra-operator reliability of 95.3% (Abrams et al. 2017a). A similar study assessed PTR-LUM's ability to detect caries in extracted teeth that were later analyzed with transverse microradiography (TMR). The authors concluded that PTR-LUM measurements were

comparable to those of TMR, suggesting that PTR-LUM was a reliable method to track enamel demineralization over time (Jeon et al. 2008).

Detecting caries beneath a sealant without identifiable marginal leakage is difficult with visual and radiographic examination, and an easy, reliable alternative technique is desired. Silvertown *et al.* (2017) compared the Canary system with DIAGNOdent to assess their relative abilities in identifying pit and fissure caries beneath opaque dental sealants. The authors selected 40 extracted teeth with or without occlusal caries, scanned them with DIAGNOdent and Canary systems, placed opaque dental sealants, and scanned them again with both instruments. The Canary System was able to distinguish between sound and carious occlusal surfaces under an opaque sealant 79% of the time, whereas DIAGNOdent was successful 59% of the time. A similar study compared the accuracy of PTR-LUM caries detection with radiographs, visual assessment, and DIAGNOdent, and found that the sensitivity was significantly higher with PTR-LUM and that specificity was the same as that for DIAGNOdent. The authors concluded that the combination of PTR-LUM was a reliable tool in diagnosing pit and fissure caries, as well as deeper lesions (Jeon et al. 2004a).

The Canary system has been shown to be a viable tool in assessing caries progression around dental restorations. In one study, the Canary system's ability to detect caries around glass ionomer and composite restorations was compared with DIAGNOdent, light emitting diode (LED) fluorescence, and the visual ICDAS. The Canary System and DIAGNOdent were able to differentiate between carious and sound tooth structure at the margins of the restorations, but the DIAGNOdent demonstrated poorer overall accuracy (Abrams et al. 2018). A similar study found the Canary System to be more accurate than other diagnostic methods in detecting caries around the margins of amalgam restorations (Abrams et al. 2017b).

Recently, researchers investigated the ability of the Canary System to detect enamel demineralization around orthodontic brackets. The authors used a glass ionomer luting cement

while bonding orthodontic brackets to see if it was able to prevent the formation of WSLs around the brackets. While the cement did not appear to significantly affect WSL formation versus the control, the Canary system appeared to be a useful diagnostic tool in tracking enamel demineralization around orthodontic brackets (Dorfman 2017).

Figure 2.1 The Canary System
The Canary System, including the laser unit (top), the operator's hand piece with plastic protective tip (bottom left) and calibration block (bottom right)

Figure 2.2 The Canary Scale
The Canary System calculates a Canary Number after scanning the tooth surface, indicating the level of decay of that tooth surface.
(https://thecanarysystem.com/about.php, retrieved on 9/18/2019, used with permission)

CHAPTER 3: SPECIFIC AIMS AND RESEARCH HYPOTHESIS

3.1 Statement of the Problem

White spot lesions are a common and detrimental side effect of orthodontic treatment in patients whose oral hygiene is not ideal. Even in patients whose oral hygiene is satisfactory prior to the placement of orthodontic appliances, once the appliances are placed, their oral hygiene quality may decrease because of increased plaque accumulation and retention on the brackets. WSLs, once present, pose a significant esthetic challenge to the dental practitioner once the braces are removed and, without irreversible and significant dental restorative procedures, may not be able to be adequately removed or camouflaged. In spite of the variety of methods that have been attempted to prevent WSLs, the only one that has been consistently shown to be effective has been adequate patient compliance with oral hygiene. Unfortunately, patient compliance in orthodontic treatment is usually lacking, especially in the adolescent age group that comprises the majority of orthodontic patients, necessitating the advent of a material that can prevent WSLs without the need for proper patient compliance. Developing such a material is critical in achieving predictable prevention of WSLs during orthodontic therapy, increased esthetic outcomes, and long-term dental health once the orthodontic appliances are removed. This research will evaluate the efficacy of a certain varnish in preventing enamel demineralization with the use of an early caries detection device.

3.2 Central Research Hypothesis

The central research hypothesis is that RMGI extended contact varnish will be effective in preventing the progression of enamel demineralization adjacent to and distant from orthodontic brackets.

3.3 Specific Aims

1) Evaluate the efficacy of a RMGI varnish to prevent enamel demineralization adjacent to orthodontic brackets
2) Determine if the efficacy of a RMGI varnish is affected by the distance from the margin of an orthodontic bracket

CHAPTER 4: MATERIALS AND METHODS

4.1 Study Design

A total of 45 human molars were obtained from a collection of discarded extracted teeth that had been stored in 1:10 sodium hypochlorite solution. The teeth were transferred to a jar of distilled water prior to sample preparation for storage. Any identifying information regarding the extracted teeth was unknown and this protocol was considered IRB-exempt. The teeth were cleaned of gross debris with distilled water and allowed to dry for scanning with an early caries detection instrument called the Canary System. The buccal and lingual surfaces of the teeth were scanned three times each in order to determine which surface had the least amount of enamel demineralization and, therefore, would be the most appropriate surface to use for this research. Tooth surfaces that were restored or exhibited pre-existing WSLs were excluded from the study. The surface demonstrating the lowest average of three Canary Numbers, with at least two of three numbers being below 25 (indicating the absence of pre-existing decay) was the surface that was used. This surface was marked with a small permanent marker dot on the root surface near the CEJ.

Once it was determined which surfaces would be scanned, the roots of the teeth were removed via coarse interproximal reduction (IPR) disc and the root canal space sealed with composite resin. The surface not being scanned was flattened and smoothed so that the surface of interest would remain in an upright position while being exposed to its respective solution and during scanning. A total of 40 teeth met inclusion criteria and had the proper Canary Numbers.

Prior to bracket cementation, the teeth were randomly assigned to four different treatment groups for a total of 10 teeth per group. The groups were as follows: (1) varnish in distilled water (V-DW), (2) varnish in acetic acid (V-AA), (3) exposed enamel in distilled water (E-DW), and (4) exposed enamel in acetic acid (E-AA). For each tooth, twin composite brackets (FLI

Composite, Rocky Mountain Orthodontics) were cemented to the teeth after application of a self-etch primer to the tooth surface (S.E.P., Reliance Orthodontics) and a composite resin to the bracket base (Brace Paste, American Orthodontics). The application of the self-etch primer was limited to the area where the bracket was to be placed and flash from the composite resin was removed as completely as possible with a 90-degree angle explorer in order to prevent any erroneous scores or change the enamel demineralization around the bracket area. The composite resin was then cured with a light-emitting diode (LED) curing light (Ortholux Luminous Curing Light, 3M, St Paul, MN) for 24 seconds, ensuring that all sides of the bracket had equal light exposure. The intensity of the blue light was measured at 1600 mW/cm^2 with a light meter that was built into the charging base of the curing light.

After the brackets were cemented, two 3 x 3 mm windows were created on the tooth surface using a fine graphite pencil. One window was created immediately adjacent to the bracket (near site = N) and a second window placed with the side closest to the bracket 3 mm away from the side of the first window that was distant from the bracket (Distant site = D) (Figure 4.1). These windows demarcated scanning sites for the Canary System.

Prior to the placement of the varnish on the teeth in the V-AA and V-DW groups, scans with the Canary System were taken at the near and distant sites. Each scan was repeated five times per site for a total of 10 scans per tooth. One layer of varnish was then applied to the entire tooth and cured with a light-emitting diode (LED) curing light (Ortholux Luminous Curing Light, 3M, St Paul, MN) for 12 seconds per buccal or lingual surface. The near and distant sites for teeth in the V-AA and V-DW groups were scanned again, five times each, to determine if the presence of the varnish changed the Canary Numbers to such an extent that they were statistically different than the Canary Numbers of the original scans. The teeth that did not receive the varnish (E-DW and E-AA groups) were scanned at both their N and D sites, five times each, for a total of 10 scans per tooth. 1M acetic acid was poured into 20 small cylindrical plastic containers (Esslinger, St.

Paul, MN) with screw-off lids, measuring 25 mm in diameter and 30 mm in height, and another 20 containers were filled with distilled water. The samples were then placed in their respective containers with the flattened surface contacting the floor of the container so that the bracketed surface was in a stable position facing upwards. The containers were placed in an incubator (Thelco Model Z, Precision Scientific) at 37°C for the duration of the experiment. The cylindrical containers were given their respective labels as follows: V-AA 1-10 for Vanish XT in acetic acid, V-DW 1-10 for Vanish XT in distilled water, E-AA 1-10 for exposed enamel in acetic acid, and E-DW 1-10 for exposed enamel in distilled water (Figure 4.2). Follow-up scans were taken at 3 days (T1), 7 days (T2), and 14 days (T3).

4.2 Canary System Scans

The Canary System uses PTR-LUM technology to determine the extent of demineralization of enamel at a specific site. It was utilized in this study to assess the level of enamel demineralization at locations adjacent to and distant from orthodontic brackets. The varnish used in this experiment was applied in a thin layer, less than 0.5mm, as per manufacturer instructions. The Canary System has been shown to be capable of detecting caries beneath opaque restorations and at depths of $\leq$ 5mm from the outer enamel surface (Abrams et al. 2017a; Silvertown et al. 2017b). The handpiece is positioned on the enamel surface and a scan activated by pressing a button (Figure 4.3). Once the scan is complete, a color-coded number is displayed on a connected computer monitor, indicating the degree of demineralization at that enamel site. Normal enamel is indicated by a green color and a score of 0-20, decay is indicated by a yellow color and a score of 21-70, and advanced decay is indicated by a red color and a score of 71-100 (Figure 2.2).

In this experiment, the samples were scanned at baseline (T0), 3 days (T1), 7 days (T2), and 14 days (T3), and 5 scans were obtained at each tooth site. Samples coated with varnish were scanned before and after varnish placement to determine if the varnish rendered any effect on the Canary Numbers. During the experiment at T1 through T3, the samples were removed from their

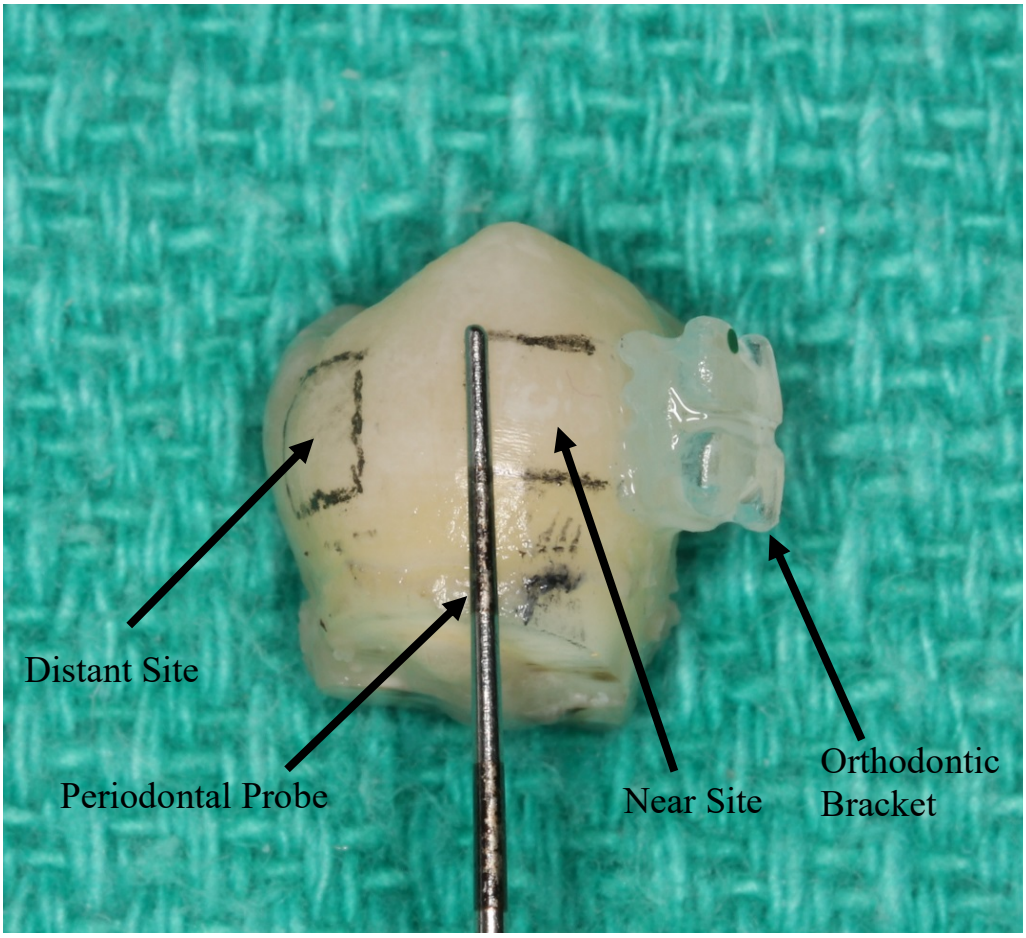

Figure 4.1 Single Tooth Sample
Example of a tooth sample with the graphite windows for the near site and the distant site inscribed on the tooth surface. The orthodontic bracket is visible on the right side of the tooth sample. The periodontal probe was used to measure the height and width of the graphite windows.

Figure 4.2 Labeled Sample Containers
Picture of labeled sample containers with their respective labels and samples

Figure 4.3 Example of Canary System Scan
Example of the Canary System handpiece scanning the buccal surface of a lower left second premolar

respective containers and dried for a few minutes before scanning. The N and D sites for each tooth were scanned 5 times each for a total of 10 scans per tooth, per time point. The tip of the Canary System handpiece was positioned within each 3 x 3 mm graphite window and held as close as possible to be flush with the tooth surface. For N sites, the handpiece tip was positioned as close as possible to the bracket. A single investigator performed all scans and recorded the Canary Numbers. After storage in distilled water for two weeks after the conclusion of the experiment, three teeth from each group were scanned five times for each near and distant site by the same investigator, in order to determine intra-rater reliability.

4.3 Sample Solutions

The control groups for this experiment consisted of samples either receiving or not receiving varnish and immersed in distilled water (V-DW and E-DW). The test groups included samples either varnished or not varnished and immersed in 1 M acetic acid (V-AA and E-AA). The solutions were manually agitated every three days for approximately one minute to remove possible buildup of degradation products on tooth surfaces, and the solutions were refreshed at T2.

4.4 Scanning Electron Micrographs

At the conclusion of the experiment, the Canary Numbers were compiled and the sample from each group at T3 whose mean for the N-site was closest to the group N-site mean was selected and prepared for imaging. Two teeth were selected from each of the two varnish groups, with one tooth immersed in ethanol for five minutes in order to remove the varnish and permit direct SEM inspection of enamel surfaces beneath the varnish. In addition, one tooth was imaged before and after the varnish was placed at T0 to provide baseline enamel and varnish surfaces for comparison to the enamel and varnish surfaces at T3. Specific teeth imaged at T3 were: V-AA #5 and #8, E-AA #4, V-DW #4 and #8, E-DW #8. Teeth with varnish removed by ethanol prior to scanning included V-DW #4 and V-AA #5. Prior to SEM examination, the teeth were sectioned

into smaller sizes for placement onto SEM stubs, placed into a vacuum desiccator with silica gel desiccant for seven days, and then placed into a vacuum incubator (Fisher Isotemp Vacuum Oven, Fisher Scientific) at 45°C under 60 psi vacuum for seven days. The samples were removed and both bracket/enamel interfaces at the N site, as well as the D site areas, were imaged using SEM (Hitachi S4700 Field Emission SEM, Hitachi Ltd.) at 1000 times and 100 times magnification, 5.0 kV, and an acceleration voltage of 5000V.

4.5 Statistical Treatment of Data

Descriptive statistics (means, standard errors) of Canary numbers were calculated for each group and a 4-way analysis of variance (ANOVA) with full interaction determined the presence of significant differences contributed by the four factors (distilled water vs. acid media, near vs. distant sites, varnished vs. unvarnished surfaces and time, $p<0.05$). Pairwise comparisons between groups were accomplished with a Tukey Kramer *post hoc* test, with the confidence limit set at 95% ($p \leq 0.05$). To determine if varnish application affected Canary results, a paired t-test was performed for Canary Scans at T0 for the V-AA and E-AA groups before and after the varnish application. To assess intra-rater reliability, both Cronbach's alpha and paired t-tests were applied to repeated measurements. All statistical analyses were performed with NCSS 2004 statistical software (NCSS, Kaysville, UT).

CHAPTER 5: RESULTS

5.1 Data Summary

Table 5.1 shows a summary of the data collected for the Canary System scans at T0, T1, T2, and T3 including mean scores, range, standard errors and overall changes in Canary Number between T0 to T3. Table 5.2 presents differences between timepoints (T0-T3), varnish treatments, location (N or D) and storage solution. Figure 5.1 is a graphical representation of the data collected with standard error bars. Four-way ANOVA (Table 5.3) and Tukey-Kramer Multiple-Comparison test (Table 5.4) indicated significant differences occurred between timepoints, varnish-no varnish treatments and acid-no acid exposures ($p<0.05$), but not between locations ($p\geq0.05$). To further confirm the significant differences between the groups, a 3-way ANOVA (Table 5.5) was performed with the locations pooled since the 4-way ANOVA indicated that there were no significant differences between scanning location. The 3-way ANOVA demonstrated significant differences between varnish treatments and acid-no acid exposures ($p<0.05$), confirming the results of the 4-way ANOVA. All interaction terms without the location term included were significant ($p<0.05$).

5.2 Effects of Storage Time

As expected, no significant difference in mean Canary scores were measured at T0. (Table 5.4). For groups stored in distilled water, mean Canary scores did not change significantly between T0 and T3 for either varnished or unvarnished surfaces. For groups exposed to acetic acid, mean Canary scores increased over time with changes from T0 to T3 of 19.5 to 38.4 for the V-AA N group, 19.0 to 39.8 for the V-AA D group, 20.2 to 62.16 for the E-AA N group, and 18.6 to 60.5 for the E-AA D group. The only exception to a continued increase of mean Canary Score over time was between T2 and T3 for the V-AA N group where the mean Canary Score decreased from 39.3 at T2 to 38.4 at T3, though this decrease was not statistically significant.

Table 5.1 Summary of Canary Scores

	T0 (Baseline)	T1 (3 days)	T2 (7 days)	T3 (14 days)	Overall Change in Canary Number (T0-T3)
	Enamel-Distilled Water (Near Site)				
Mean	20.7	19.6	19.6	17.9	-2.8
Range	12 (15-27)	17 (12-29)	16 (12-28)	15 (10-25)	
Standard Error	0.700	0.833	1.157	0.900	
	Varnish-Distilled Water (Near Site)				
Mean	20.6	21.0	20.3	19.8	-0.8
Range	11 (16-27)	11 (16-27)	14 (14-28)	14 (13-27)	
Standard Error	0.702	0.596	0.684	0.593	
	Varnish-Acetic Acid (Near Site)				
Mean	19.5	30.4	39.3	38.4	18.9
Range	15 (10-25)	26 (15-41)	25 (28-53)	33 (20-53)	
Standard Error	0.654	1.628	1.745	2.377	
	Enamel-Acetic Acid (Near Site)				
Mean	20.2	33.6	43.5	62.2	42.0
Range	18 (11-29)	17 (24-41)	25 (32-57)	30 (45-75)	
Standard Error	0.867	0.777	1.79	2.185	
	Enamel-Distilled Water (Distant Site)				
Mean	19.1	19.2	17.6	18.9	-0.2
Range	16 (11-27)	10 (15-25)	15 (12-27)	15 (12-27)	
Standard Error	0.983	0.629	0.542	0.433	
	Varnish-Distilled Water (Distant Site)				
Mean	18.5	18.3	18.1	17.7	-0.8
Range	15 (11-26)	14 (12-26)	17 (11-28)	16 (9-25)	
Standard Error	0.719	0.667	0.795	0.761	
	Varnish-Acetic Acid (Distant Site)				
Mean	19.0	30.8	34.5	39.8	20.8
Range	16 (12-28)	29 (15-44)	35 (17-52)	25 (26-51)	
Standard Error	0.882	2.581	2.952	1.919	
	Enamel-Acetic Acid (Distant Site)				
Mean	18.6	33.0	44.2	60.5	41.9
Range	10 (12-22)	20 (24-44)	19 (32-51)	32 (43-75)	
Standard Error	0.306	1.506	0.904	1.833	

Table 5.2 Average Canary Scores T0-T3

Time	Near Site				Distant Site			
	Varnish - Distilled Water	**Enamel - Distilled Water**	**Varnish - Acetic Acid**	**Enamel - Acetic Acid**	**Varnish - Distilled Water**	**Enamel - Distilled Water**	**Varnish - Acetic Acid**	**Enamel - Acetic Acid**
T0 (0 Days)	20.6^{a}	20.7^{a}	19.5^{a}	20.2^{a}	18.5^{a}	19.1^{a}	19.0^{a}	18.6^{a}
T1 (3 Days)	21.0^{a}	19.6^{a}	30.4^{b}	33.64^{bc}	18.3^{a}	19.2^{a}	30.8^{b}	33.02^{bc}
T2 (7 Days)	20.3^{a}	19.6^{a}	39.3^{cd}	43.5^{d}	18.1^{a}	17.6^{a}	34.5^{c}	44.2^{d}
T3 (14 Days)	19.8^{a}	17.9^{a}	38.4^{cd}	62.16^{e}	17.7^{a}	18.9^{a}	39.8^{cd}	60.5^{e}

Average Canary Scores per group. Groups with a same superscript letter are not significantly different at the alpha = 0.05 level of confidence. Group comparisons are both vertical and horizontal.

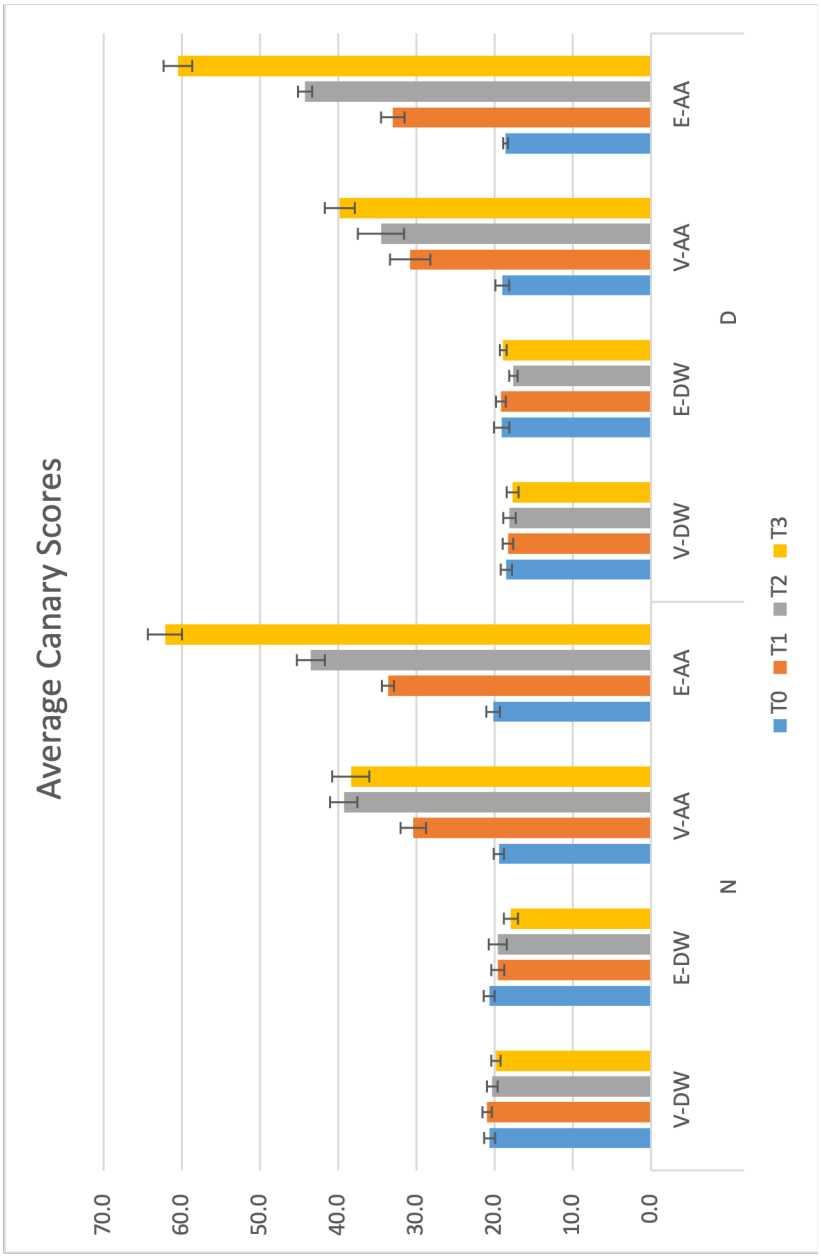

Figure 5.1 Average Canary Numbers T0-T3
Bar graphs demonstrating each group's mean±s.e. Canary Score, with groups divided into Near (N) and Distant (D) scanning sites.

Table 5.3 Four-Way ANOVA Table

Analysis of Variance Table

Source Term	DF	Sum of Squares	Mean Square	F-Ratio	Prob Level	Power (Alpha=0.05)
A: Timepoint	3	9501.075	3167.025	179.37	0.000000*	1.000000
B: Varnish	1	1216.8	1216.8	68.92	0.000000*	1.000000
AB	3	1414.125	471.375	26.70	0.000000*	1.000000
C: Location	1	110.45	110.45	6.26	0.129360	0.702919
AC	3	33.775	11.25833	0.64	0.591316	0.183220
BC	1	12.8	12.8	0.72	0.395231	0.135695
ABC	3	30.375	10.125	0.57	0.632893	0.168216
D: Acid	1	21222.61	21222.61	1201.99	0.000000*	1.000000
AD	3	11126.16	3708.721	210.05	0.000000*	1.000000
BD	1	1353.012	1353.012	76.63	0.000000*	1.000000
ABD	3	1529.512	509.8375	28.88	0.000000*	1.000000
CD	1	9.1125	9.1125	0.52	0.473090	0.110484
ACD	3	5.4125	1.804167	0.10	0.958722	0.068380
BCD	1	10.5125	10.5125	0.60	0.440972	0.120020
ABCD	3	89.4625	29.82083	1.69	0.169502	0.440405
S	288	5085	17.65625			
Total (Adjusted)	319	52750.2				
Total	320					

Table 5.4 Tukey-Kramer Multiple-Comparison Test for Main Effects

Group	Count	Mean	Different From Groups
T0	80	19.525	T1, T2, T3
T1	80	25.7375	T0, T2, T3
T2	80	29.6375	T0, T1, T3
T3	80	34.4	T0, T1, T2

Group	Count	Mean	Different From Groups
D	160	26.7375	
N	160	27.9125	

Group	Count	Mean	Different From Groups
Varnish	160	25.375	No Varnish
No Varnish	160	29.275	Varnish

Group	Count	Mean	Different From Groups
No Acid	160	19.18125	Acid
Acid	160	35.46875	No Acid

Table 5.5 Three-Way ANOVA Table with Location Pooled

Analysis of Variance Table

Source Term	DF	Sum of Squares	Mean Square	F-Ratio	Prob Level	Power (Alpha=0.05)
A: Timepoint	3	9501.075	3167.025	178.73	0.000000*	1.000000
B: Varnish	1	1216.8	1216.8	68.67	0.000000*	1.000000
AB	3	1414.125	471.375	26.60	0.000000*	1.000000
C: Acid	1	21222.61	21222.61	1197.66	0.000000*	1.000000
AC	3	11126.16	3708.721	209.29	0.000000*	1.000000
BC	1	1353.012	1353.012	76.35	0.000000*	1.000000
ABC	3	1529.512	509.8375	28.77	0.000000*	1.000000
S	304	5386.9	17.72007			
Total (Adjusted)	319	52750.2				
Total	320					

* Term significant at alpha = 0.05

5.3 Varnish Placement Effects

Prior to measuring Canary numbers in test groups, Canary system was assessed in its ability to maintain consistent scores before and after varnish placement at the same location. Results from paired t-tests demonstrated that the scores were not significantly different from before and after varnish placement for all groups ($0.48<p<0.96$) except for the acid-exposed varnished group, near site, where varnish placement produced an increase in the mean Canary Score of 1.3 (18.1 to 19.5) ($p=0.02$).

Groups immersed in acetic acid experienced a significant increase in mean Canary Scores between T0 and T3 with ranges of 42.0 for the E-AA N group, 41.9 for the E-AA D group, 20.8 for the V-AA D group, and 18.9 for the V-AA N group, which were all statistically significant ($p<0.05$). At T1 and T2, all groups placed in acetic acid, regardless of whether or not varnish was applied, exhibited a significant increase in mean Canary Scores, which indicated the presence of demineralization ($p>0.05$). However, the mean Canary Scores for groups V-AA N and V-AA D at T3 were 38.4 and 39.8, respectively, which were significantly lower ($p<0.05$) than the mean Canary Scores for groups E-AA N and E-AA D, which were 62.16 and 60.5, respectively. The near-site mean Canary Score for the acid-exposed varnished group at T3 was 38.4, which was slightly lower than the mean Canary Score at T2 (39.3) but this decrease from T2 to T3 was not statistically significant.

5.4 Location Effects

For all groups, there were no significant differences in mean Canary scores between near and distant sites at all time points ($p>0.05$), as confirmed by four-way ANOVA and Tukey-Kramer multiple comparison analyses (Table 5.3, 5.4) and further confirmed by three-way ANOVA with locations pooled (Table 5.5).

5.5 Intra-Rater Reliability

Intra-rater reliability was assessed by first evaluating if there were any significant differences between the original and repeat scans of the samples with a paired t-test. At an alpha of 0.05 there were no significant differences between the original and repeat measurements. Then, to assess the internal consistency of the original and repeat measurements, a Cronbach's alpha test yielded a value of 0.997, demonstrating high intra-rater reliability.

5.6 Scanning Electron Microscope Images

The enamel surface at T0 was scanned before and after the varnish was placed to establish a baseline appearance of enamel and varnish (Figs 5.2, 5.3). The unvarnished enamel surface under SEM presented with small pits and other irregularities interspersed along the surface. The surface of the varnish under SEM demonstrated a rough, particulate surface with particles of varying sizes.

The samples that were scanned under SEM at T3 were varnish and acid-exposed (#5 and #6), unvarnished and acid-exposed (#4), varnished stored in distilled water (#4 and #8) and unvarnished stored in distilled water, (#8). Each sample was scanned at both near and distant sites.

Figures 5.4 and 5.5 show the near and distant sites for Varnish-Acetic Acid #5 at T3, following varnish removal with ethanol. For both sites, the enamel surfaces had similar appearances. The enamel appearances for both sites were also similar to that of the SEM images taken of enamel at T0 (Fig 5.2) though the enamel prisms observed at T0 were not obviously present in the varnished, acid-exposed tooth sample.

Figures 5.6 and 5.7 show near and distant sites for Varnish-Acetic Acid #6 at T3, where the varnish was retained. The varnished surface of figure 5.6 was disrupted and demonstrated significant cracking and a small portion of the enamel surface was visible under the varnish. In

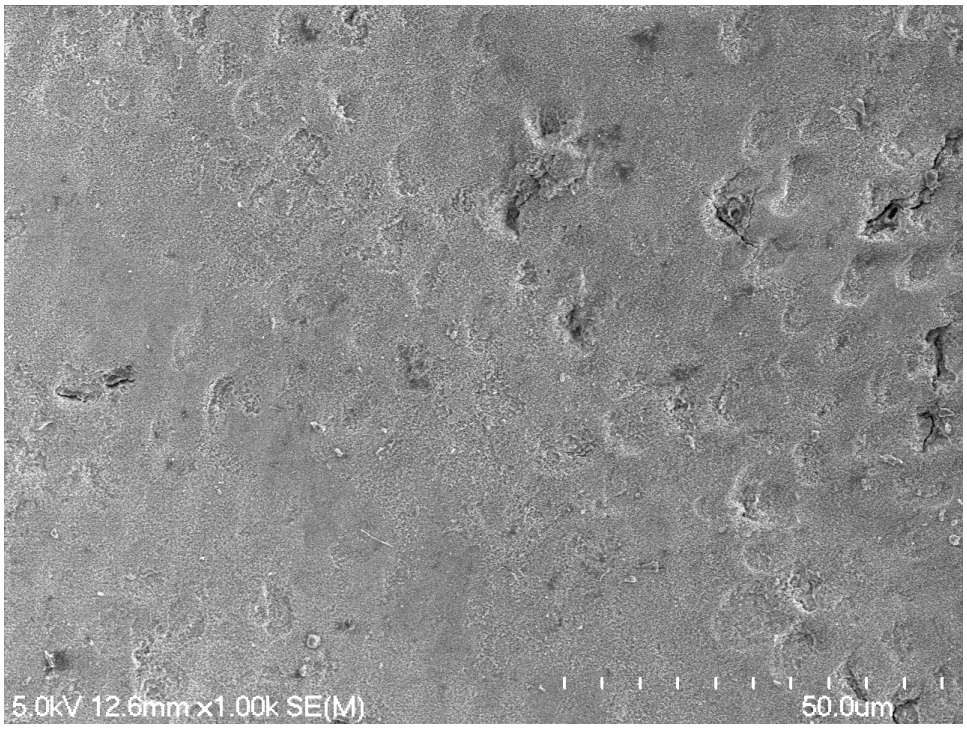

Figure 5.2 SEM Image of Unvarnished Enamel Surface at T0 (1000x Magnification)
The enamel surface demonstrates a regular pattern of circular enamel prisms with some interspersed pitting

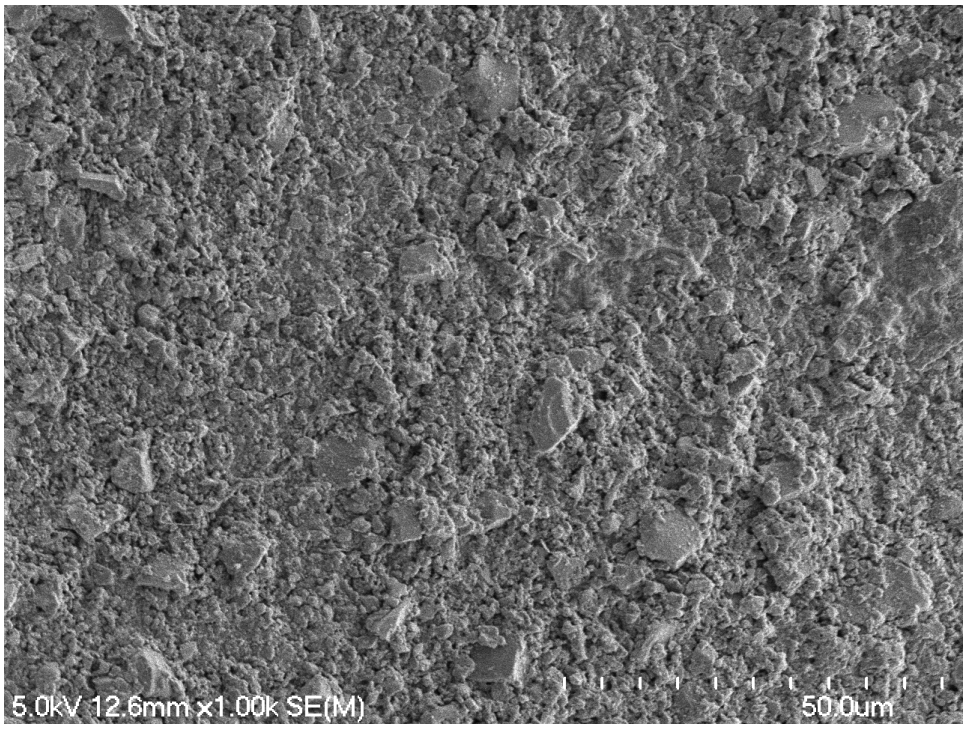

Figure 5.3 SEM Image of Varnished Enamel Surface at T0 (1000x Magnification)
The surface demonstrates a rough particulate surface with particles of varying sizes.

figure 5.7, the varnished surface appeared to have significant grooving and cracking and there was some exposed enamel visible as well, which appeared to have a rough surface with some cracking or grooving present. The varnish surfaces were different than the varnish surface at T0, which did not exhibit cracking in the material. The enamel surfaces appeared to be rougher and more pitted than the enamel at T0.

Figures 5.8 and 5.9 show near and distant sites for Enamel-Acetic Acid #4. The enamel surfaces at both sites demonstrated significant enamel cratering and pitting, which was not observed in the enamel surface at T0 or the enamel surface at T3 for the V-AA groups (Figs 5.4, 5.5). It is noteworthy that upon preparation for SEM inspection, the enamel began to shear away from the dentin while in the vacuum incubator, demonstrating a significant level of surface demineralization.

Figures 5.10 and 5.11 show near and distant sites for Varnish-Distilled Water #4 at T3, which had the varnish removed with ethanol prior to imaging. The enamel surface for both sites demonstrated the enamel prisms, which appeared very similar to the enamel prisms observed at T0 (Fig 5.2). The similar appearance of the enamel prisms suggested that demineralization had not occurred.

Figures 5.12 and 5.13 show the near and distant sites for Varnish-Distilled Water #8, which had the varnish retained for imaging. The surface of the varnish at both sites demonstrated visible cracking within the material, though the distant site demonstrated more significant cracking. This is in contrast to the absence of cracking observed in figure 5.3. Cracking of the material was also observed in V-AA #6 (Figs 5.6, 5.7) at the near and distant sites, but the cracking was more distinct in V-DW #8 (Figs 5.12, 5.13).

Figures 5.14 and 5.15 show the near and distant sites for Enamel-Distilled Water #8.The top of the enamel prisms are visible and have a similar appearance to the enamel prisms at T0 (Fig 5.2). There is some additional pitting observed in the enamel surfaces at both the near and

distant sites that are not visible in the enamel surface at T0 (Fig 5.2), however, the pitting is significantly less than the pitting and cratering noted in the enamel surface for E-AA #8 (Figs 5.8, 5.9).

The margin of the bracket and varnish was evaluated with SEM at T3 to detect possible gap presence that could potentially permit acid diffusion at the bracket margin. Fig. 5.6 demonstrates the appearance of the varnish-bracket interface. Although there is significant disruption in the varnish surface, the original bracket-varnish interface is still visible in the image and it does not appear that there is an appreciable gap in the interface.

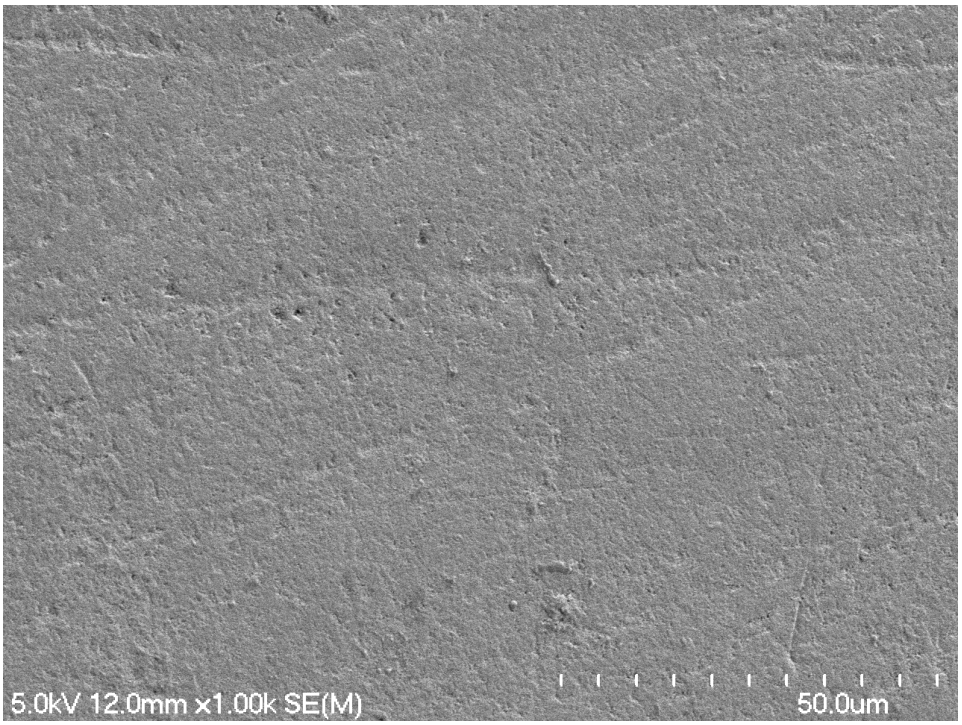

Figure 5.4 SEM Image of Varnished and Acid-Exposed Enamel Surface #5, Near Site, with Varnish Removed at T3 (1000X Magnification)
The surface demonstrates small, interspersed pitting and grooving with no visible enamel prism pattern as that seen in figure 5.2

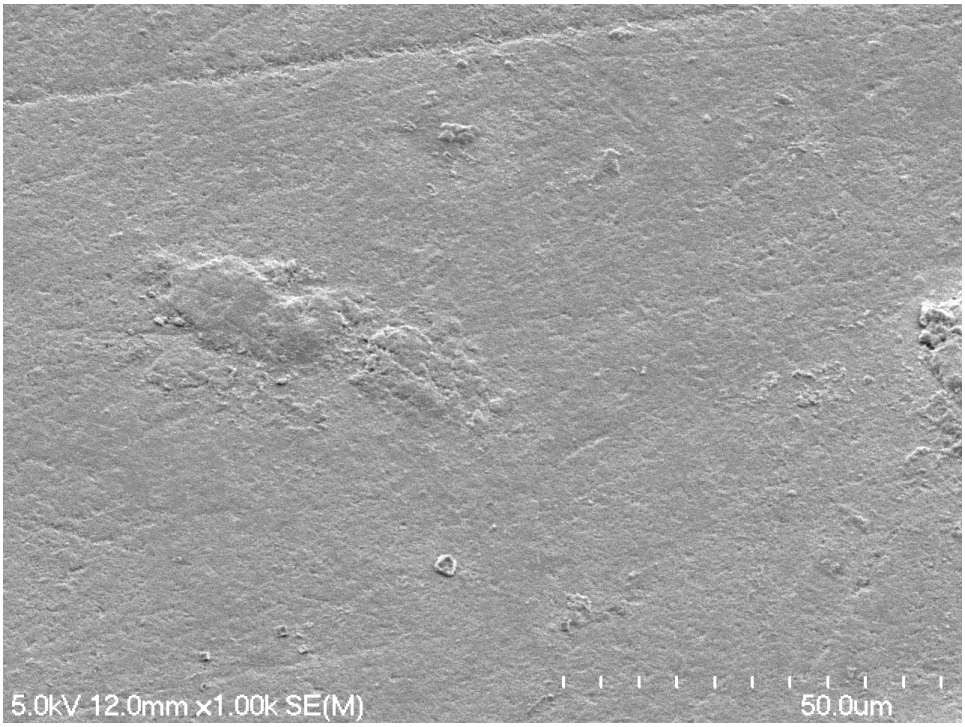

Figure 5.5 SEM Image of Varnished and Acid-Exposed Enamel Surface #5, Distant Site, with Varnish Removed at T3 (1000X Magnification)
The surface demonstrates interspersed small indentations and grooving without distinguishable enamel prism pattern

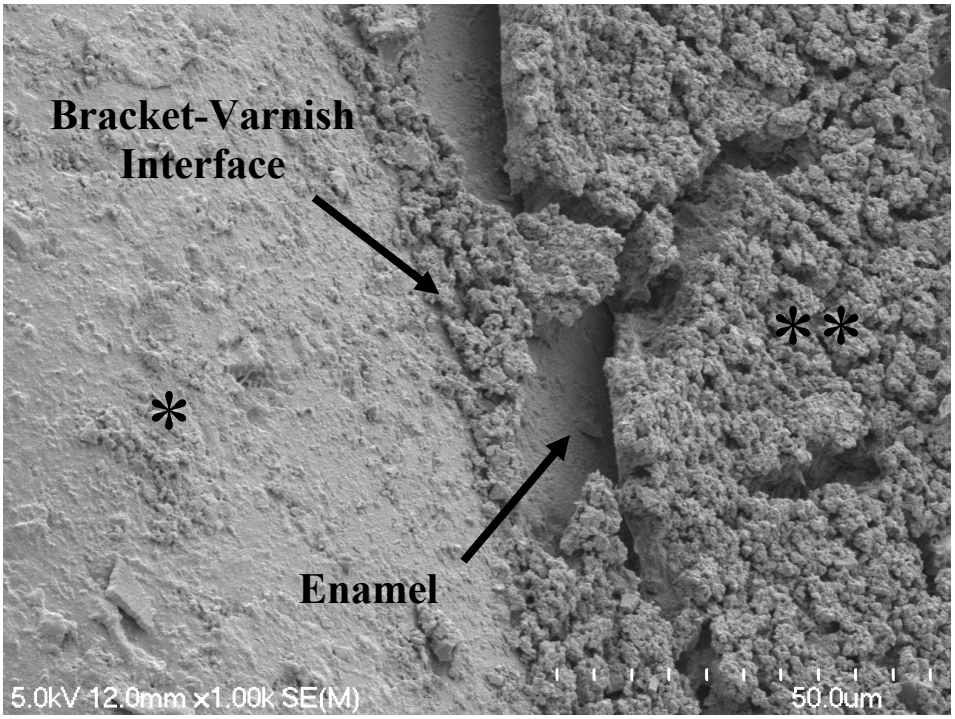

Figure 5.6 SEM Image of Acid-Exposed Varnish Surface #6, Near Site, at T3 (1000X Magnification)
Orthodontic bracket surface (*) adjacent to disrupted varnish layer (**) with small amount of enamel surface visible underneath the varnish layer (arrow). Also, the bracket-varnish interface is visible in this view (arrow) without an apparent gap between the two surfaces.

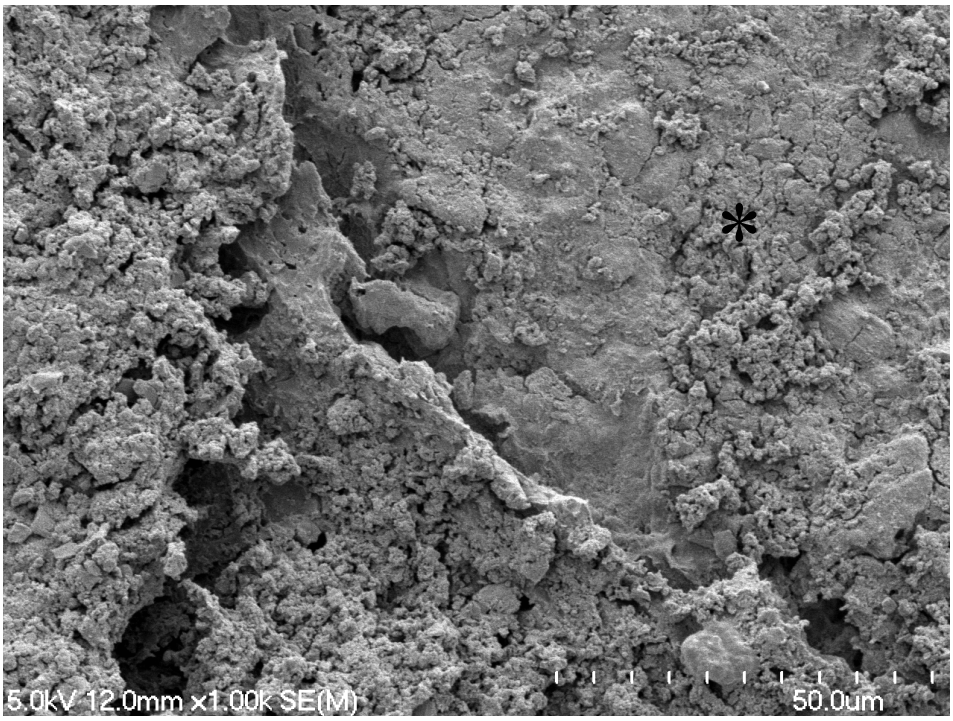

Figure 5.7 SEM Image of Acid-Exposed Varnish Surface #6, Distant Site, at T3 (1000X Magnification)
The surface of the varnish demonstrates a rough, particulate surface with grooving and cracking and with exposed enamel (*) in the upper right-hand corner of the image that appears to have a small amount of cracking and pitting with a rougher surface than the control enamel.

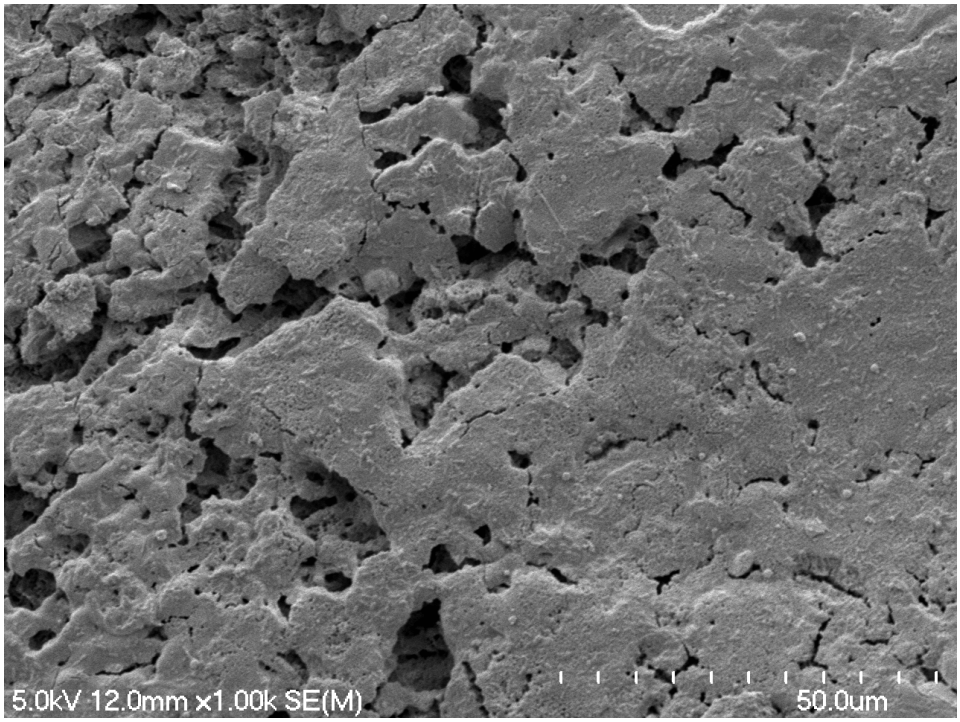

Figure 5.8 SEM Image of Acid-Exposed Enamel Surface #4, Near Site, at T3 (1000X Magnification)
The enamel surface demonstrates significant cratering and pitting

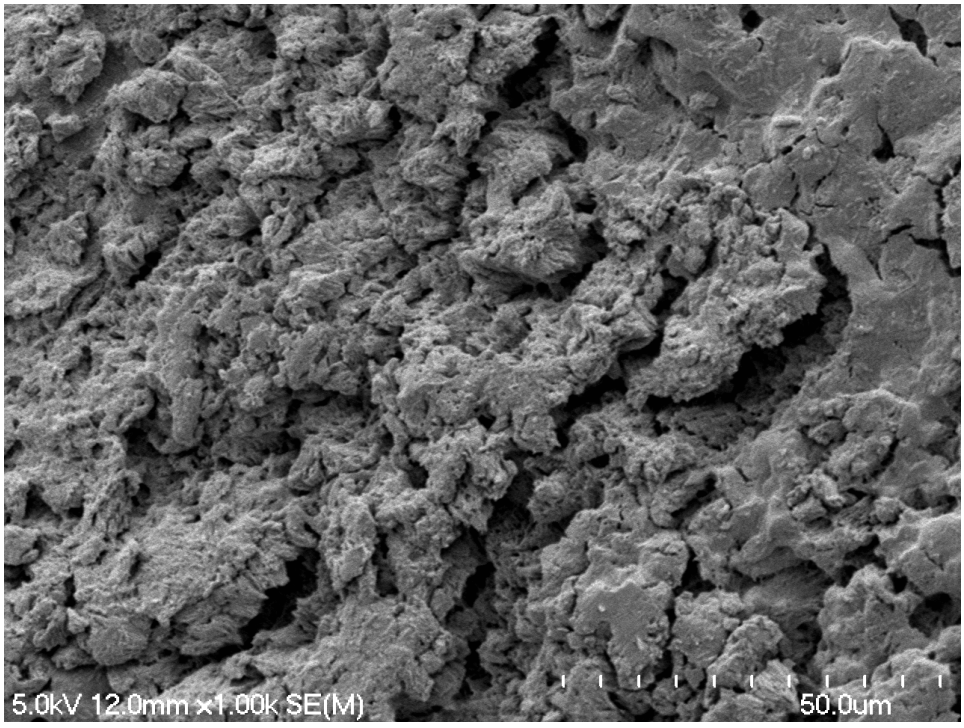

Figure 5.9 SEM Image of Acid-Exposed Enamel Surface #4, Distant Site, at T3 (1000X Magnification)
The enamel surface demonstrates significant cratering and pitting to a greater extent than in figure 5.8

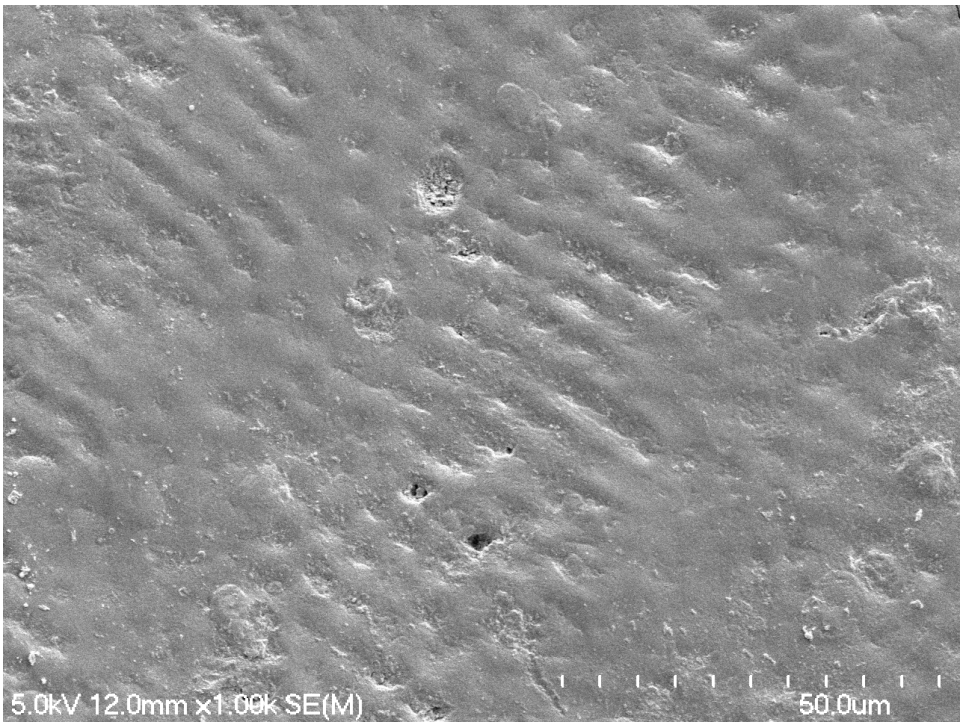

Figure 5.10 SEM Image of Varnished and Distilled-Water-Exposed Enamel Surface #4, Near Site, with Varnish Removed at T3 (1000X Magnification)
The enamel surface demonstrates a pattern of circular enamel prism indentations

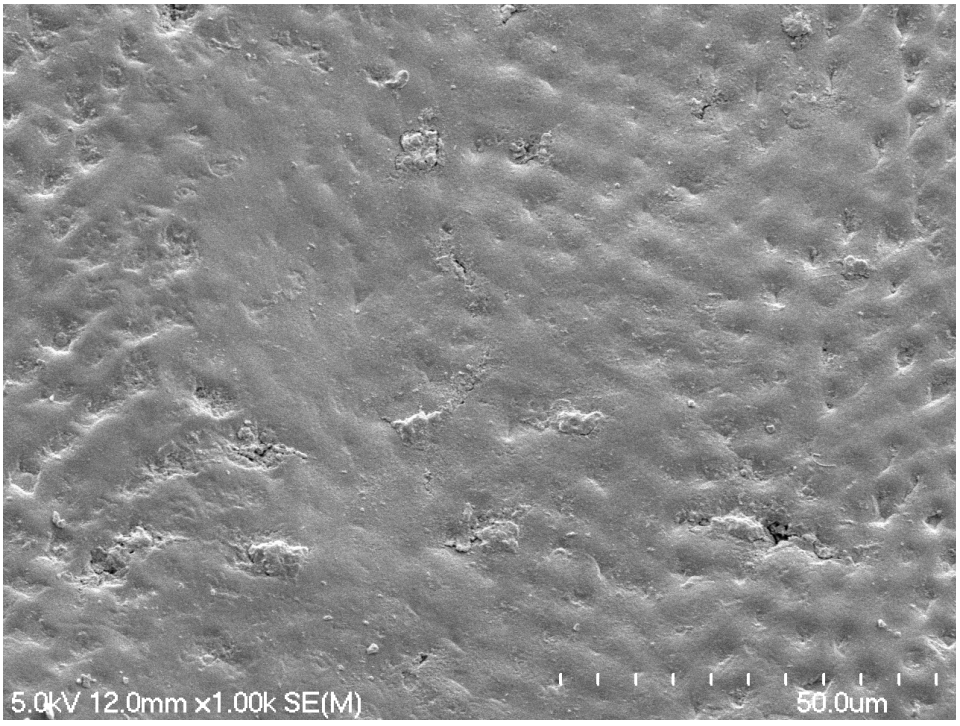

Figure 5.11 SEM Image of Varnished and Distilled-Water-Exposed Enamel Surface #4, Distant Site, with Varnish Removed at T3 (1000X Magnification

The enamel surface demonstrates a pattern of circular enamel prism indentations

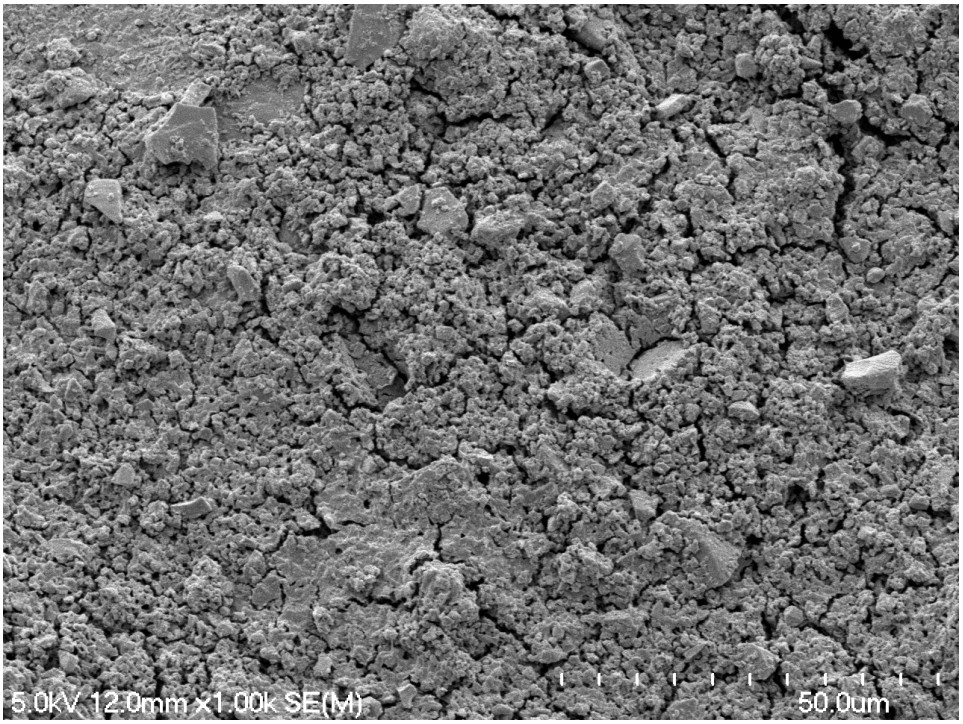

Figure 5.12 SEM Image of Distilled-Water-Exposed Varnish Surface #8, Near Site, at T3 (1000X Magnification)
The surface demonstrates a rough, granular appearance with cracking

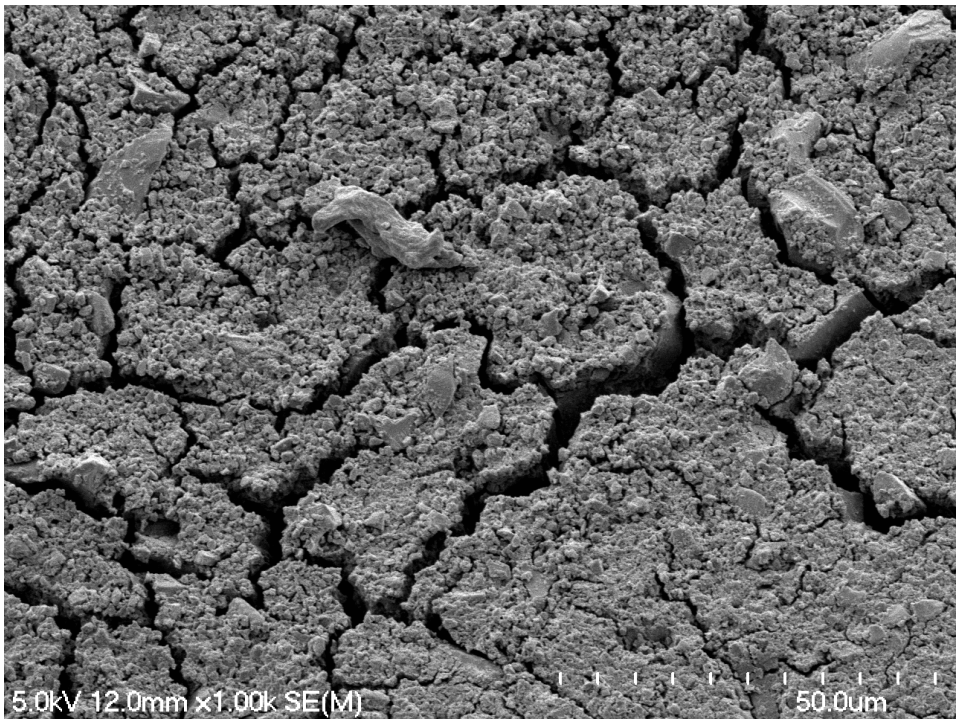

Figure 5.13 SEM Image of Distilled-Water-Exposed Varnish Surface #8, Distant Site, at T3 (1000X Magnification)
The surface demonstrates a rough, granular surface with more cracking than seen in Figure 5.12

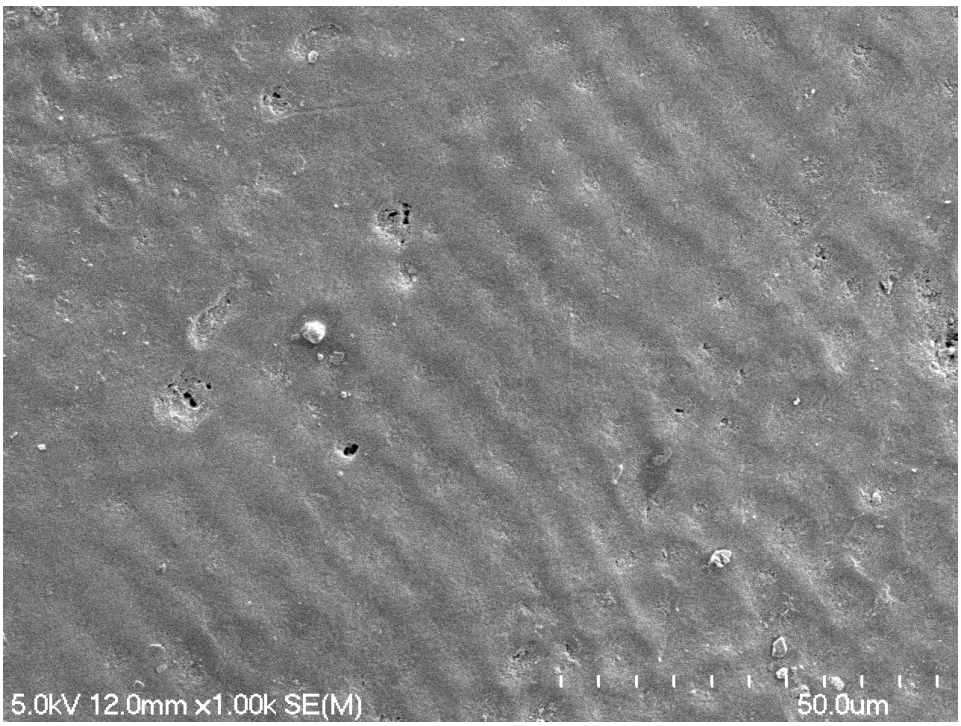

Figure 5.14 SEM Image of Distilled-Water-Exposed Enamel Surface #8, Near Site, at T3 (1000X Magnification)
The enamel surface demonstrates a pattern of circular enamel prism indentations with some interspersed pitting

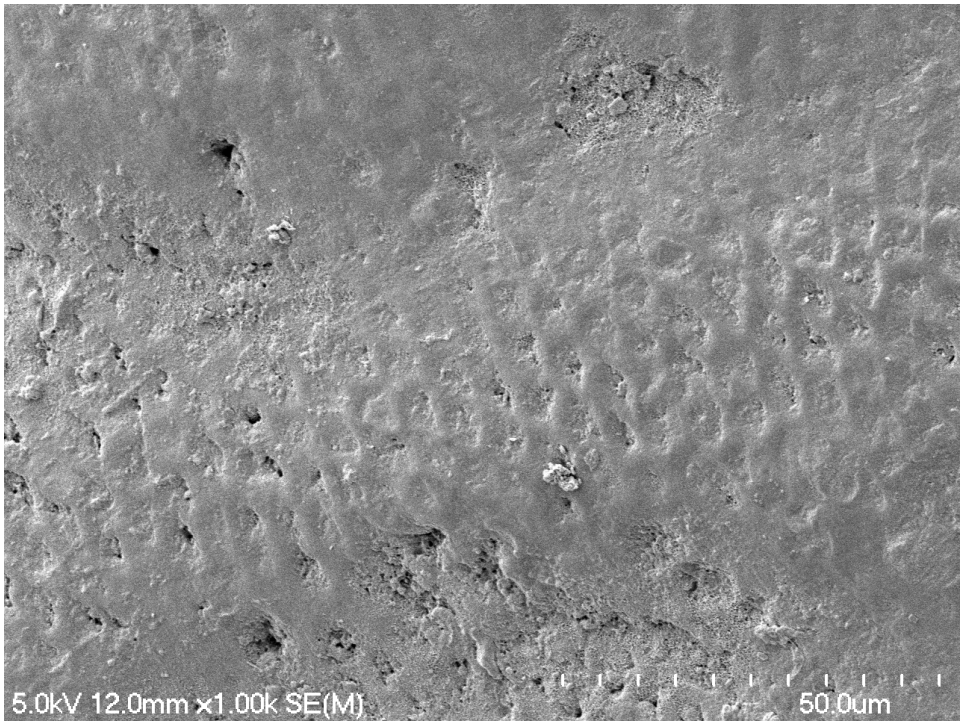

Figure 5.15 SEM Image of Distilled-Water-Exposed Enamel Surface #8, Distant Site, at T3 (1000X Magnification)
The enamel surface demonstrates a pattern of circular enamel prism indentations with some interspersed pitting

CHAPTER 6: DISCUSSION

6.1 Varnish Effects

The development of WSLs in orthodontic patients is a common problem that orthodontists face on a daily basis. The development of WSLs is preventable with proper oral hygiene, but, unfortunately, many orthodontic patients are non-compliant, as the nature of orthodontic appliances makes oral hygiene difficult. This study evaluated the ability of a resin-modified glass ionomer (RMGI) varnish to prevent enamel demineralization around orthodontic brackets in the presence of an acid challenge. The primary hypothesis of this study was that the varnish would be able to prevent enamel demineralization at sites near to and distant from the orthodontic bracket margin. Enamel demineralization was assessed indirectly at both sites using the PTR-LUM technology of the Canary System. While the results indicated that the varnish was protective from demineralization caused by acid challenge, the protective effect was not demonstrated by the Canary Score until the time interval between T2 and T3. This means that the presence of the varnish did not start to protect the enamel from demineralization until it had been exposed to an acid challenge for a prolonged time period (i.e. 2 weeks). Proximity to the orthodontic bracket was not a factor, as mean Canary scores for near and distant sites were similar ($p \geq 0.05$)

The mean Canary Scores for the V-AA group had an increase of approximately 15-20 from T0 to T2 but did not significantly increase from T2 to T3. This initial increase in Canary Score was also seen the E-AA group, though to a greater extent. This may be explained by the nature of the fluoride release from the varnish. The maximum fluoride release of this varnish at any given timepoint has been shown to be less than 0.5 ppm, but the release extends over a six-week period (Jablonowski et al. 2012). For initial time points (T1 and T2), the Canary Score increase for the V-AA group may be because an inadequate amount of time had lapsed for a sufficient amount of cumulative fluoride to be released and adequately protect the enamel surface

from the acid challenge. With no additional score increases from T2 to T3, cumulative fluoride levels may have been sufficient to protect the enamel surface. These results suggest that enamel protection offered by the varnish may be limited soon after its placement, but more effective over extended time periods.

The increase of 15-20 in the Canary score for the V-AA group is somewhat concerning because this increase in magnitude places the enamel surface into the decay category on the Canary scale. It may be necessary to couple the varnish with another fluoride-releasing material to provide initial protection following bracket placement.

The SEM images of enamel surfaces coated with varnish demonstrated significantly less pitting than those with uncoated surfaces, suggesting the varnish's effectiveness in preventing enamel demineralization. In figures 5.4 and 5.5, the varnished and acid-exposed enamel surface with varnish removed prior to imaging demonstrated enamel surface characteristics similar to that of the control enamel (Figs 5.14, 5.15), although with interspersed pitting and without clearly defined enamel prisms. This indicates that some enamel demineralization may have occurred, although not to the degree as unvarnished acid-exposed surfaces (Figs 5.8, 5.9). Those surfaces demonstrated significant cratering, suggesting the presence of severe enamel demineralization.

SEM images of enamel surfaces appeared to correspond with Canary scores at T3 for all groups. Enamel surfaces of control groups (those immersed in distilled water) demonstrated similar appearances to enamel surfaces observed at T0 (Fig 5.2). Enamel surfaces of control groups demonstrated regular patterns of enamel prisms (Figs 5.10, 5.11, 5.14, 5.15) with minimal interspersed pitting. Likewise, mean Canary scores at all time points for control groups were maintained within a range of healthy/sound tooth structure, indicating that demineralization was not occurring on the enamel surface. With varnish present, the varnish layer demonstrated significant cracking (Fig 5.6 and 5.7), with minimal debonding from enamel. Since Canary scores were similar between T2 and T3, it is likely that varnish cracking was a result of SEM

preparation, where samples were dried at elevated temperature. Surface desiccation of glass ionomer materials is known to induce crazing, which is consistent with these observations. With control samples stored in distilled water demonstrating similar coating cracking (Figs 5.12, 5.13), the phenomenon is considered as artifact.

In the SEM images for unvarnished enamel that was exposed to acetic acid, significant enamel erosion and cratering was observed (Figs 5.8, 5.9). The enamel appearance suggested that significant demineralization occurred, which was consistent with the significant increase in the mean Canary scores for this group. The mean Canary Score increased from 18.6 to 60.5 for the distant site and from 20.2 to 62.16 for the near site. A Canary score of 60 is close to the upper limit of the decay category for the Canary Scale and approaches the level of advanced decay (Figure 2.2). Mean Canary scores for the varnished, acid-exposed group stabilized between 34.5 and 39.3, while the mean Canary Scores for the non-varnished, acid-exposed group did not stabilize and continued to increase.

The results of this study regarding the varnish's ability to prevent WSLs is in agreement with the limited studies available in the literature regarding this particular varnish. In 2018, Wiewora et al demonstrated that Vanish XT was able to prevent the development of WSLs adjacent to orthodontic brackets when compared to controls. Other studies evaluating the ability of fluoride-releasing varnishes to prevent WSLs have found them to be effective in the prevention of enamel demineralization (Kumar Jena et al. 2015; Salar et al. 2007).

In a study by Kaur et al. (2017), orthodontic patients planned for premolar extraction received a fluoride varnish on twenty teeth. Twenty premolars without varnish treatment served as controls. After two months, the premolars were extracted and the area of enamel near the orthodontic bracket was imaged under SEM. The Vickers microhardness testing was also performed to compare the SEM appearance of the enamel with microhardness. Enamel with fluoride varnish applied demonstrated several eroded areas near the enamel-bracket interface,

which corresponded to a minimal decrease in microhardness. This suggested minimal enamel demineralization occurred. By contrast, enamel surfaces of control teeth exemplified significant erosion (Kaur et al. 2017). The authors' results are similar to those presented in this study, as remarkable differences in erosion can be seen on varnished and unvarnished surfaces under acid challenge (Figs 5.4, 5.5 vs. 5.8, 5.9).

An *in vitro* study by Premaraj et al. (2017), assessed the fluoride release and ability to resist acid penetration of two different fluoride-releasing varnishes. For fluoride release, the results indicated that there was adequate short-term fluoride release from the two varnishes to prevent demineralization, but the fluoride release decreased significantly over the course of 28 days. The authors suggested that the fluoride would potentially need to be recharged for long-term prevention of demineralization. This seems somewhat contradictory to this study as the short-term increased fluoride release should have kept the mean Canary Scores low until the fluoride release decreased, but this study demonstrated the opposite. This is likely due to the extended-release nature of the Vanish XT. As mentioned previously, the maximum short-term fluoride release from Vanish XT is lower, but is released over an extended period of time (Jablonowski et al. 2012). It is feasible that the fluoride release of Vanish XT has a lag time between the initial acid attack and when the fluoride concentration has reached a sufficient level to prevent demineralization. This would explain the pattern of mean Canary Scores that were observed for the varnished, acid-exposed group.

Premaraj et al. (2017) also assessed the ability of the varnishes to prevent acid penetration utilizing SEM and the Knoop hardness test. The SEM images for the non-varnished enamel surfaces demonstrated significant destruction of the enamel surface and this correlated with the microhardness testing (Premaraj et al. 2017). The SEM images in the present study also demonstrated significant damage to the non-varnished, acid-exposed enamel surfaces and much less damage to the varnished acid-exposed enamel surfaces.

As stated previously, the acid-exposed varnish group experienced an initial increase in Canary score, which stabilized over time. This suggested that a time lapse occurred before enamel protection was achieved. These results differ from those reported by Wiewora et al. (2018), where Vanish XT effectively prevented WSL formation on bracketed teeth immersed in 0.1 M lactic acid for 32 days. These differences could be attributed to the different data collection timepoints in both studies. This study assessed enamel demineralization at 3, 7, and 14 days, while the study by Wiewora et al. first assessed the enamel demineralization after 32 days. In both studies, varnish effectiveness was observed after an extended period of time, but in this study, the short-term effects were also assessed. Had the study by Wiewora et al. assessed the short-term effects in addition to the long-term effects, their results may have been different.

Fluorescence microscopy and visual assessment was used in the study by Wiewora et al. (2018) to evaluate enamel surfaces, while this study utilized PTR-LUM to assess both surface and subsurface enamel. Fluorescence microscopy utilizes a laser that is directed onto a sample surface, which is then reflected off the sample surface by the fluorescing components of the sample. PTR-LUM also utilizes a laser, but as the laser is emitted into the enamel surface and it is reflected back to the scanning unit, the reflection and absorption of the laser light by the enamel structure is analyzed rather than the fluorescence. In addition, PTR-LUM analyzes the thermal response of the tooth to the laser, which fluorescence microscopy does not. Since fluorescence microscopy does not measure subsurface damage, the extent of the demineralization can be underestimated. In addition, visual assessments can only determine the presence or absence of WSLs and not the actual level of enamel demineralization, which may be subtle and not appear clinically until a significant amount of enamel demineralization has occurred. These differences in measurement may explain the differences in reported varnish effectiveness.

In contrast to the results of this study, some studies have found that the use of a varnish is not effective in the prevention of WSLs (Hammad and Knosel 2016; Wenderoth et al. 1999).

Hammad and Knosel (2016) used an organo-selenium varnish and Wenderoth et al. (1999) used a lightly filled Bis-GMA sealant, both of which likely do not exhibit the same fluoride release of a resin-modified glass ionomer varnish. The organo-selenium varnish utilized selenium as an antimicrobial to prevent bacterial biofilm formation and hence acid release. The Bis-GMA sealant did exhibit some fluoride release, but it was likely immediate and reduced rapidly in contrast to glass ionomer varnish which exhibits fluoride release over a more extended period of time. These different properties likely explain the conflicting results between these studies and the one reported here, as the Bis-GMA and organo-selenium varnishes may not be as effective as other fluoride-releasing varnishes. In addition, Hammad and Knosel (2016) noted that although the organo-selenium varnish alone was not sufficient to prevent WSLs, it could be effective when used in conjunction with other preventative measures such as proper oral hygiene.

6.2 Bracket Proximity

Based on the results of this study, the level of enamel demineralization was independent of the location of the enamel surface relative to the margin of the orthodontic bracket. Based on the statistical analysis, both distant and near sites on the same tooth displayed similar amounts of demineralization for the acetic acid groups. This suggests that the varnish is able to create a tight junction, or overlap, between the margin of the bracket or the adhesive and the varnish itself, preventing acid penetration between the margins of the two materials. This was verified visually via SEM imaging directed at the interface between the bracket margin and varnish (Fig 5.6).

6.3 Canary Score

The ability of the Canary System to detect and track enamel demineralization was also evaluated in this study. First of all, the effect of the varnish on the Canary score was evaluated and it was found that, overall, the varnish exhibited a protective effect, but only after a period of time. When scans were taken at T0 before and after varnish placement, the differences in mean Canary scores within three of the four groups (V-DW D, V-DW N, V-AA D) were not

significant, but they were significantly different within the V-AA N group (p=0.02). The exact cause for this discrepancy is unknown, but since the three groups demonstrated negligible varnish effects, it is likely due to positioning error, especially since the score only increased 1.3 units between pre and post varnish. An increase of 1.3 is likely not clinically significant and possibly below the resolution of the measuring instrument. Other *in vitro* research has demonstrated the Canary System's ability to accurately detect enamel demineralization at depths of $\leq$ 5mm from the enamel surface and under opaque dental sealants (Abrams et al. 2017a; Silvertown et al. 2017b) suggesting that positioning error is more likely to be responsible for the statistically significant differences in Canary Scores for the V-AA N group.

Second, the ability of the Canary System to detect enamel demineralization over time was subjectively assessed by comparing the Canary Score at T3 with the visual appearance of the enamel under SEM at the same time point. At T3 the mean Canary Score for groups E-AA and V-AA indicated that severe demineralization had occurred on the unvarnished enamel surface and that more moderate demineralization occurred on enamel protected by varnish. Based on the SEM images, it was apparent that the E-AA group had undergone significant enamel demineralization with the presence of deep pitting of the enamel surface. In contrast, the enamel surface of the V-AA group demonstrated interspersed pitting. The mean Canary Scores for the control groups (V-DW and E-DW) remained statistically the same within and between groups for the duration of the experiment, and this was corroborated by visual assessment under SEM. Enamel surfaces of the control groups appeared similar to the enamel surface at T0. It was interesting that the V-AA enamel surface did not exhibit additional pitting when the Canary System indicated a moderate level of demineralization. This is possible because the Canary System analyzes the surface and subsurface enamel quantitatively and detects subtle changes in the enamel structure, whereas SEM is a subjective, visual assessment of only the enamel surface. Although there may be significant changes in the Canary score, these changes may not be visually apparent until more

significant demineralization has occurred. This is in keeping with the current literature that has concluded that radiographs and other visual assessment modalities may not detect subtle enamel demineralization as well as PTR-LUM (Jeon et al. 2004a; Jeon et al. 2008; Jeon et al. 2004b). In addition, a study by Dorfman, J (2017) demonstrated that the Canary Score correlated well with the visual appearance of demineralized enamel with photomicrographs.

6.4 Limitations

As with any research study, limitations were present that may have affected the final results of this study. Although the intra-rater reliability for this study was high, it would have been beneficial to have involved multiple examiners to better mitigate any operator-specific errors. Also, blinding was unable to be performed as the varnish was readily apparent on the tooth surface and the examiner that performed the measurements also applied the varnish to the teeth. It would have been better to have had multiple examiners who were blinded to the treatment received by each tooth and formulate a placebo varnish to apply to the control teeth.

One important limitation of this study was that the angulation of the laser relative to the enamel surface was not able to be controlled. This most likely introduced some variability into the Canary Scores as the enamel structure from one scan at one angulation to another scan at another angulation is likely different although the relative scanning site is the same. In order to eliminate any angulation issues, a stabilization jig for the Canary handpiece would have been helpful. However, the Canary System is primarily a clinical instrument and so it is to be expected that angulations will vary from scan to scan in clinical use. Thus, the scanning methodology used in this study may better approximate actual clinical performance versus using a stabilizing jig.

Another limitation was that there was no way to standardize the enamel structure from one tooth to the next since extracted teeth were used. Even if multiple teeth in the study were from the same patient, there was no way to know this due to the de-identified nature of the teeth. Furthermore, enamel structure and environmental modification of the enamel over time (e.g.

fluoride exposure and level of acid challenge) from patient to patient is variable, which could potentially leave some patients more susceptible to demineralization than others. Although each tooth was scanned with the Canary System at T0 to ascertain the baseline level of demineralization, the demineralization rates could have been variable among teeth due to differences in their enamel structures. Using a standardized enamel model or a much larger sample size would help mitigate the effect of individual tooth differences.

Another limitation of this study was that only the enamel surface was evaluated, and that the evaluation was qualitative in nature. It would have been beneficial to have had quantifiable testing that takes subsurface enamel demineralization into account in order to determine the level of enamel demineralization. This would have allowed for a more quantifiable assessment of the accuracy of the Canary Scores in detecting enamel demineralization. Also, preparing samples for scanning electron microscopy (SEM) requires desiccation and incubation under vacuum. During sample processing for SEM, the surface of the enamel and varnish could have been altered from their original appearance. This was especially a concern with the varnish surface, as it exhibited cracking at T3, and it was unclear whether the cracking was due to the vacuum incubation and desiccation or the prolonged acid challenge. In order to clarify this uncertainty, it would have been beneficial to have taken an impression of the samples prior to processing for SEM to capture the surface condition of the varnish and enamel. The impression could then have been poured up in die stone and evaluated under SEM to determine the surface condition of the enamel and varnish prior to sample processing.

One final limitation of this study is the translatability of this *in vitro* study to clinical practice. The oral cavity involves complex interactions of teeth, tissues, saliva, and other materials that are difficult, if not impossible, to replicate precisely in *in vitro* experimentation. While the manner in which the teeth were scanned closely approximated clinical conditions, other factors such as salivary composition and quantity were not taken into consideration.

6.5 Conclusions

Based on the results obtained in this study, it can be concluded that Vanish XT is an effective adjunct modality for the prevention of WSLs in orthodontic patients, especially those with a high caries risk and prolonged oral acidity. The mean Canary Score differences between the varnish and non-varnish groups that were exposed to acetic acid indicated that there was significantly higher demineralization in the group that did not have the varnish applied to the enamel surface. For T2 and T1, these differences were not significant, also suggesting that the varnish may not be as beneficial in the prevention of WSLs in patients at a moderate or low caries risk or good oral hygiene compliance.

It was hypothesized that the distance of the enamel surface from the bracket may be significant in the level of demineralization. More specifically, that more enamel demineralization may be present at the bracket margin due to the presence of incomplete closure of the varnish-bracket margin interface. However, this was not supported by the data or the SEM imaging. Therefore, it can be concluded that the location of the enamel relative to the bracket margin is not a significant factor in enamel demineralization.

Finally, based on the data and the SEM images, the Canary System was found to be a reliable instrument in detecting enamel demineralization and tracking its progression over time. When the Canary Scores were compared at T0 between V-DW N, D and V-AA N, D, before and after the varnish was placed, three of the four group measurements did not have statistically significant differences. In the group that did have significant differences, angulation variation was most likely the cause of the differences, and the actual difference in mean Canary Scores was unlikely to be of clinical significance. When comparing the enamel surface appearance under SEM at T0 and T3 versus the Canary scores at those time points, the Canary score appeared to be consistent with the condition of the enamel. Therefore, based on the data in this study, the Canary

System is a useful instrument in detecting enamel demineralization and tracking its progression over time in orthodontic patients.

6.6 Future Research

Future research on this topic could include looking at the demineralization of each tooth with the Canary System and verifying it with a diagnostic method capable of measuring subsurface and surface demineralization with higher accuracy. This would provide a more accurate scale from which Canary scores can be interpreted.

Another future topic of research could be assessing the ability of the Canary System to detect carious lesions around orthodontic brackets of different materials. It is feasible that the more reflective orthodontic bracket materials (i.e. stainless steel) could have a substantial effect on the accuracy of the Canary Scores. This type of research would be helpful to have available for the orthodontic practitioner who is considering using the Canary System as a diagnostic tool since the brackets that are used by the practitioner may be influencing the Canary Score.

As long as orthodontic patients demonstrate a lack of compliance with their oral hygiene regimens, there will always be a need for the development of materials or devices that are effective in preventing WSLs. While many devices and materials have been marketed for detecting and preventing WSLs, more reliable, sensitive detectors and long-lasting caries-preventive materials are still needed.

BIBLIOGRAPHY

3M. 2008. Vanish xt technical profile brochure.

Abrams SH, Sivagurunathan KS, Silvertown JD, Wong B, Hellen A, Mandelis A, Hellen WMP, Elman GI, Mathew SM, Mensinkai PK et al. 2017a. Correlation with caries lesion depth of the canary system, diagnodent and icdas ii. Open Dent J. 11:679-689.

Abrams T, Abrams S, Sivagurunathan K, Moravan V, Hellen W, Elman G, Amaechi B, Mandelis A. 2018. Detection of caries around resin-modified glass ionomer and compomer restorations using four different modalities in vitro. Dent J (Basel). 6(3).

Abrams TE, Abrams SH, Sivagurunathan KS, Silvertown JD, Hellen WMP, Elman GI, Amaechi BT. 2017b. In vitro detection of caries around amalgam restorations using four different modalities. Open Dent J. 11:609-620.

Basdra EK, Huber H, Komposch G. 1996. Fluoride released from orthodontic bonding agents alters the enamel surface and inhibits enamel demineralization in vitro. Am J Orthod Dentofacial Orthop. 109(5):466-472.

Chen H, Liu X, Dai J, Jiang Z, Guo T, Ding Y. 2013. Effect of remineralizing agents on white spot lesions after orthodontic treatment: A systematic review. Am J Orthod Dentofacial Orthop. 143(3):376-382 e373.

Corbett JA, Brown LR, Keene HJ, Horton IM. 1981. Comparison of streptococcus mutans concentrations in non-banded and banded orthodontic patients. J Dent Res. 60(12):1936-1942.

Derks A, Kuijpers-Jagtman AM, Frencken JE, Van't Hof MA, Katsaros C. 2007. Caries preventive measures used in orthodontic practices: An evidence-based decision? Am J Orthod Dentofacial Orthop. 132(2):165-170.

Dorfman JM. 2017. Cement composition effects on enamel demineralization adjacent to orthodontic brackets: An in vitro study using the canary system [M.S.]. [Ann Arbor]: Temple University.

Ekstrand KR, Gimenez T, Ferreira FR, Mendes FM, Braga MM. 2018. The international caries detection and assessment system - icdas: A systematic review. Caries Res. 52(5):406-419.

Fejerskov O, Kidd EAM. 2003. Dental caries : The disease and its clinical management. Oxford ; Malden, MA: Blackwell.

Fournier A, Payant L, Bouclin R. 1998. Adherence of streptococcus mutans to orthodontic brackets. Am J Orthod Dentofacial Orthop. 114(4):414-417.

Geiger AM, Gorelick L, Gwinnett AJ, Griswold PG. 1988. The effect of a fluoride program on white spot formation during orthodontic treatment. Am J Orthod Dentofacial Orthop. 93(1):29-37.

Gorelick L, Geiger AM, Gwinnett AJ. 1982. Incidence of white spot formation after bonding and banding. Am J Orthod. 81(2):93-98.

Gorton J, Featherstone JD. 2003. In vivo inhibition of demineralization around orthodontic brackets. Am J Orthod Dentofacial Orthop. 123(1):10-14.

Hammad SM, Knosel M. 2016. Efficacy of a new sealant to prevent white spot lesions during fixed orthodontic treatment : A 12-month, single-center, randomized controlled clinical trial. Journal of orofacial orthopedics = Fortschritte der Kieferorthopadie : Organ/official journal Deutsche Gesellschaft fur Kieferorthopadie. 77(6):439-445.

Heymann GC, Grauer D. 2013. A contemporary review of white spot lesions in orthodontics. Journal of esthetic and restorative dentistry : official publication of the American Academy of Esthetic Dentistry [et al]. 25(2):85-95.

Ho CS, Ming Y, Foong KW, Rosa V, Thuyen T, Seneviratne CJ. 2017. Streptococcus mutans forms xylitol-resistant biofilm on excess adhesive flash in novel ex-vivo orthodontic bracket model. Am J Orthod Dentofacial Orthop. 151(4):669-677.

Jablonowski BL, Bartoloni JA, Hensley DM, Vandewalle KS. 2012. Fluoride release from newly marketed fluoride varnishes. Quintessence international (Berlin, Germany : 1985). 43(3):221-228.

Jeon RJ, Han C, Mandelis A, Sanchez V, Abrams SH. 2004a. Diagnosis of pit and fissure caries using frequency-domain infrared photothermal radiometry and modulated laser luminescence. Caries Res. 38(6):497-513.

Jeon RJ, Hellen A, Matvienko A, Mandelis A, Abrams SH, Amaechi BT. 2008. In vitro detection and quantification of enamel and root caries using infrared photothermal radiometry and modulated luminescence. J Biomed Opt. 13(3):034025.

Jeon RJ, Mandelis A, Sanchez V, Abrams SH. 2004b. Nonintrusive, noncontacting frequency-domain photothermal radiometry and luminescence depth profilometry of carious and artificial subsurface lesions in human teeth. J Biomed Opt. 9(4):804-819.

Jeon RJ, Matvienko A, Mandelis A, Abrams SH, Amaechi BT, Kulkarni G. 2007. Detection of interproximal demineralized lesions on human teeth in vitro using frequency-domain infrared photothermal radiometry and modulated luminescence. J Biomed Opt. 12(3):034028.

Kaur T, Tripathi T, Rai P, Kanase A. 2017. Sem evaluation of enamel surface changes and enamel microhardness around orthodontic brackets after application of co2 laser, er,cr:Ysgg laser and fluoride varnish: An in vivo study. J Clin Diagn Res. 11(9):ZC59-ZC63.

Kirschneck C, Christl JJ, Reicheneder C, Proff P. 2016. Efficacy of fluoride varnish for preventing white spot lesions and gingivitis during orthodontic treatment with fixed appliances-a prospective randomized controlled trial. Clin Oral Investig. 20(9):2371-2378.

Kumar Jena A, Pal Singh S, Kumar Utreja A. 2015. Efficacy of resin-modified glass ionomer cement varnish in the prevention of white spot lesions during comprehensive orthodontic treatment: A split-mouth study. J Orthod. 42(3):200-207.

Lundstrom F, Krasse B. 1987. Streptococcus mutans and lactobacilli frequency in orthodontic patients; the effect of chlorhexidine treatments. Eur J Orthod. 9(2):109-116.

Manton DJ. 2013. Diagnosis of the early carious lesion. Aust Dent J. 58 Suppl 1:35-39.

Mei L, Busscher HJ, van der Mei HC, Ren Y. 2011. Influence of surface roughness on streptococcal adhesion forces to composite resins. Dent Mater. 27(8):770-778.

Millett DT, Nunn JH, Welbury RR, Gordon PH. 1999. Decalcification in relation to brackets bonded with glass ionomer cement or a resin adhesive. Angle Orthod. 69(1):65-70.

Mitchell L. 1992. An investigation into the effect of a fluoride releasing adhesive on the prevalence of enamel surface changes associated with directly bonded orthodontic attachments. Br J Orthod. 19(3):207-214.

Mizrahi E. 1983. Surface distribution of enamel opacities following orthodontic treatment. Am J Orthod. 84(4):323-331.

Nakajo K, Imazato S, Takahashi Y, Kiba W, Ebisu S, Takahashi N. 2009. Fluoride released from glass-ionomer cement is responsible to inhibit the acid production of caries-related oral streptococci. Dent Mater. 25(6):703-708.

O'Reilly ME. 1987. The legal process in malpractice cases. Focus Crit Care. 14(6):67-69.

O'Reilly MM, Featherstone JD. 1987. Demineralization and remineralization around orthodontic appliances: An in vivo study. Am J Orthod Dentofacial Orthop. 92(1):33-40.

Ogaard B, Rezk-Lega F, Ruben J, Arends J. 1992. Cariostatic effect and fluoride release from a visible light-curing adhesive for bonding of orthodontic brackets. Am J Orthod Dentofacial Orthop. 101(4):303-307.

Ogaard B, Rolla G, Arends J. 1988. Orthodontic appliances and enamel demineralization. Part 1. Lesion development. Am J Orthod Dentofacial Orthop. 94(1):68-73.

Polat O, Gokcelik A, Arman A, Arhun N. 2008. A comparison of white spot lesion formation between a self-ligating bracket and a conventional preadjusted straight wire bracket. World J Orthod. 9(2):e46-50.

Premaraj TS, Rohani N, Covey D, Premaraj S. 2017. In vitro evaluation of surface properties of pro seal((r)) and opal((r)) seal(tm) in preventing white spot lesions. Orthod Craniofac Res. 20 Suppl 1:134-138.

Salar DV, Garcia-Godoy F, Flaitz CM, Hicks MJ. 2007. Potential inhibition of demineralization in vitro by fluoride-releasing sealants. Journal of the American Dental Association (1939). 138(4):502-506.

Shi XQ, Tranaeus S, Angmar-Mansson B. 2001. Comparison of qlf and diagnodent for quantification of smooth surface caries. Caries Res. 35(1):21-26.

Silvertown JD, Abrams SH, Sivagurunathan KS, Kennedy J, Jeon J, Mandelis A, Hellen A, Hellen W, Elman G, Ehrlich R et al. 2017a. Multi-centre clinical evaluation of photothermal radiometry and luminescence correlated with international benchmarks for caries detection. Open Dent J. 11:636-647.

Silvertown JD, Wong BPY, Abrams SH, Sivagurunathan KS, Mathews SM, Amaechi BT. 2017b. Comparison of the canary system and diagnodent for the in vitro detection of caries under opaque dental sealants. J Investig Clin Dent. 8(4).

Stecksen-Blicks C, Renfors G, Oscarson ND, Bergstrand F, Twetman S. 2007. Caries-preventive effectiveness of a fluoride varnish: A randomized controlled trial in adolescents with fixed orthodontic appliances. Caries Res. 41(6):455-459.

Sundararaj D, Venkatachalapathy S, Tandon A, Pereira A. 2015. Critical evaluation of incidence and prevalence of white spot lesions during fixed orthodontic appliance treatment: A meta-analysis. J Int Soc Prev Community Dent. 5(6):433-439.

Tassery H, Levallois B, Terrer E, Manton DJ, Otsuki M, Koubi S, Gugnani N, Panayotov I, Jacquot B, Cuisinier F et al. 2013. Use of new minimum intervention dentistry technologies in caries management. Aust Dent J. 58 Suppl 1:40-59.

Turkkahraman H, Sayin MO, Bozkurt FY, Yetkin Z, Kaya S, Onal S. 2005. Archwire ligation techniques, microbial colonization, and periodontal status in orthodontically treated patients. Angle Orthod. 75(2):231-236.

Voss A, Hickel R, Molkner S. 1993. In vivo bonding of orthodontic brackets with glass ionomer cement. Angle Orthod. 63(2):149-153.

Wenderoth CJ, Weinstein M, Borislow AJ. 1999. Effectiveness of a fluoride-releasing sealant in reducing decalcification during orthodontic treatment. Am J Orthod Dentofacial Orthop. 116(6):629-634.

Wiewiora C, Armbruster P, Lallier T, Ballard R. 2018. Effectiveness of vanish xt in reducing the development of white spot lesions: An in vitro study. Oral Health Prev Dent. 16(4):345-350.

Zandona AF, Zero DT. 2006. Diagnostic tools for early caries detection. Journal of the American Dental Association (1939). 137(12):1675-1684; quiz 1730.

APPENDICES

APPENDIX A: Initial Canary Scans for Buccal and Lingual Surfaces

V-AA	Surface	1	2	3	Surface	1	2	3
1	B	19	14	33	L	25	19	24
2	B	18	23	33	L	23	14	13
3	B	16	18	28	L	14	15	23
4	B	28	26	19	L	14	16	17
5	B	33	17	28	L	21	15	18
6	B	14	26	20	L	26	38	41
7	B	18	23	17	L	15	34	9
8	B	15	22	24	L	15	19	22
9	B	20	17	16	L	15	17	14
20	B	20	19	26	L	17	16	15

E-AA	Surface	1	2	3	Surface	1	2	3
1	B	27	22	26	L	29	18	15
2	B	30	19	20	L	17	14	28
3	B	32	27	28	L	16	16	20
4	B	31	26	39	L	25	22	32
5	B	20	22	26	L	23	46	45
6	B	27	19	17	L	19	27	19
7	B	25	17	23	L	14	26	24
8	B	17	19	25	L	22	21	16
9	B	31	23	19	L	20	16	11
10	B	23	15	18	L	15	15	16

V-DW	Surface	1	2	3	Surface	1	2	3
1	B	27	24	16	L	18	13	9
2	B	23	13	20	L	15	12	8
3	B	34	16	23	L	20	15	12
4	B	19	18	11	L	20	20	13
5	B	20	15	32	L	15	27	39
6	B	20	27	20	L	28	26	28
7	B	17	16	15	L	27	16	13
8	B	27	16	19	L	15	16	16
9	B	30	26	29	L	20	17	16
10	B	28	23	18	L	17	17	15

E-DW	Surface	1	2	3	Surface	1	2	3
1	B	18	14	14	L	32	18	22
2	B	23	18	22	L	30	14	16
3	B	16	20	19	L	31	20	20
4	B	19	18	14	L	27	18	17
5	B	29	16	23	L	26	28	31
6	B	19	22	9	L	11	14	19
7	B	59	29	22	L	22	25	30
8	B	19	20	27	L	16	16	17
9	B	17	28	28	L	21	13	16
10	B	20	24	34	L	21	32	40

DW = Distilled Water
AA = Acetic Acid
V = Varnish
E = Enamel/No Varnish
B = Buccal
L = Lingual

APPENDIX B: Baseline Canary Scans at T0

	V-DW (Pre-Varnish)				
	N Scans				
Sample #	**1**	**2**	**3**	**4**	**5**
1	23	24	20	23	20
2	18	21	19	20	25
3	24	25	21	19	20
4	15	15	14	19	22
5	19	20	18	17	20
6	20	20	24	21	18
7	19	16	20	23	24
8	18	18	23	20	18
9	17	19	20	26	16
10	18	16	24	19	24

	D Scans				
Sample #	**1**	**2**	**3**	**4**	**5**
1	19	22	19	19	18
2	17	15	16	17	14
3	17	20	23	19	19
4	16	20	16	19	20
5	26	25	21	20	21
6	21	16	17	19	21
7	15	18	14	14	16
8	20	18	20	17	16
9	18	24	17	26	16
10	21	17	15	14	15

	V-DW (Post-Varnish)				
	N Scans				
Sample #	**1**	**2**	**3**	**4**	**5**
1	17	18	19	17	18
2	23	27	20	19	27
3	19	19	19	19	16
4	18	19	23	19	18
5	20	23	27	25	25
6	20	25	20	18	18
7	22	19	19	19	19

8	19	20	21	26	27
9	27	24	20	18	23
10	23	16	19	19	17

	D Scans				
Sample #	**1**	**2**	**3**	**4**	**5**
1	14	15	20	16	14
2	15	15	18	14	17
3	25	20	16	22	17
4	11	20	17	16	15
5	18	19	20	20	24
6	20	17	18	13	19
7	16	22	26	18	21
8	19	24	18	20	25
9	23	17	24	20	23
10	17	18	17	15	17

	E-DW				
	N Scans				
Sample #	**1**	**2**	**3**	**4**	**5**
1	17	20	20	21	18
2	21	22	20	20	24
3	22	15	19	19	19
4	21	19	17	23	16
5	25	22	18	26	19
6	16	21	24	24	27
7	25	23	23	25	26
8	17	16	17	17	24
9	22	23	27	25	25
10	22	20	20	16	17

	D Scans				
Sample #	**1**	**2**	**3**	**4**	**5**
1	16	20	19	18	15
2	19	25	27	20	26
3	12	13	12	11	15
4	16	19	20	18	17
5	22	18	15	17	26
6	19	18	13	19	16

7	17	18	19	25	27
8	21	22	19	22	20
9	16	18	19	15	18
10	24	19	19	25	27

	V-AA (Pre-Varnish)				
	N Scans				
Sample #	**1**	**2**	**3**	**4**	**5**
1	21	20	21	19	19
2	20	18	14	15	18
3	20	16	13	19	17
4	19	19	22	17	19
5	16	19	24	18	25
6	17	19	18	17	18
7	14	15	15	17	21
8	15	14	18	17	12
9	25	27	21	24	15
10	18	16	15	19	18

	D Scans				
Sample #	**1**	**2**	**3**	**4**	**5**
1	20	15	18	19	18
2	14	15	15	15	17
3	14	19	19	20	17
4	17	16	19	19	19
5	14	16	19	18	22
6	20	20	25	23	26
7	11	14	15	17	24
8	23	17	20	17	19
9	24	20	22	22	21
10	16	14	15	16	23

	V-AA (Post-Varnish)				
	N Scans				
Sample #	**1**	**2**	**3**	**4**	**5**
1	23	19	21	17	23
2	19	16	16	17	21
3	20	19	17	17	20
4	16	25	20	20	20

5	22	21	24	25	22
6	19	23	23	21	24
7	10	18	18	15	22
8	19	17	19	16	16
9	23	23	18	19	18
10	15	18	19	22	18

	D Scans				
Sample #	**1**	**2**	**3**	**4**	**5**
1	18	19	28	22	21
2	19	19	20	15	19
3	13	18	16	19	20
4	16	19	18	18	19
5	22	20	22	16	19
6	20	21	20	24	27
7	18	20	15	19	12
8	15	15	12	14	14
9	22	25	19	23	25
10	20	23	17	19	17

	E-AA				
	N Scans				
Sample #	**1**	**2**	**3**	**4**	**5**
1	15	20	18	20	20
2	28	29	25	26	24
3	19	20	19	20	19
4	19	19	19	17	18
5	25	18	21	25	20
6	22	20	20	24	21
7	18	12	11	19	20
8	21	20	19	25	16
9	22	21	17	17	19
10	20	25	25	19	22

	D Scans				
Sample #	**1**	**2**	**3**	**4**	**5**
1	19	15	17	20	19
2	15	20	21	21	22
3	15	16	22	20	20

4	19	25	18	20	19
5	16	17	21	20	19
6	22	22	16	19	18
7	20	18	15	20	18
8	21	14	12	19	21
9	20	20	17	19	14
10	21	16	12	20	22

DW = Distilled Water
AA = Acetic Acid
V = Varnish
E = Enamel/No Varnish
N = Near
D = Distant

APPENDIX C: Canary Scans at T1

	V-DW				
	N Scans				
Sample #	**1**	**2**	**3**	**4**	**5**
1	19	24	24	20	19
2	20	26	25	18	16
3	18	19	20	18	20
4	18	18	20	19	20
5	19	22	25	26	27
6	19	24	20	23	17
7	17	22	18	25	27
8	18	19	17	20	23
9	20	24	26	25	25
10	23	18	19	20	19

	D Scans				
Sample #	**1**	**2**	**3**	**4**	**5**
1	12	15	14	16	17
2	17	18	18	21	24
3	18	21	23	23	26
4	14	16	15	16	17
5	21	17	15	17	16
6	16	19	21	17	22
7	15	20	15	17	16
8	22	21	20	18	16
9	19	19	16	20	24
10	13	19	20	18	22

	E-DW				
	N Scans				
Sample #	**1**	**2**	**3**	**4**	**5**
1	12	20	16	18	16
2	17	18	20	19	19
3	20	20	24	20	19
4	20	21	19	17	23
5	24	19	20	15	20
6	15	14	20	15	14
7	20	17	17	17	19

8	17	19	21	24	26
9	15	20	17	23	23
10	24	25	25	21	29

	D Scans				
Sample #	**1**	**2**	**3**	**4**	**5**
1	19	17	22	16	23
2	15	16	16	19	19
3	15	16	16	19	19
4	18	19	16	17	19
5	19	23	25	25	23
6	16	18	19	19	18
7	20	18	19	19	16
8	17	20	20	23	25
9	18	22	20	20	19
10	19	19	22	23	23

	V-AA				
	N Scans				
Sample #	**1**	**2**	**3**	**4**	**5**
1	25	26	27	28	27
2	26	25	28	18	15
3	31	26	24	20	24
4	23	31	32	30	32
5	24	27	28	30	24
6	33	31	32	34	36
7	33	31	32	34	36
8	37	39	39	40	41
9	34	34	35	38	32
10	37	29	30	35	34

	D Scans				
Sample #	**1**	**2**	**3**	**4**	**5**
1	27	23	22	28	27
2	15	19	19	16	27
3	25	18	18	18	23
4	30	24	27	30	28
5	34	33	34	32	33
6	41	38	40	40	44

7	41	38	40	40	44
8	29	31	35	37	28
9	26	29	30	29	30
10	38	39	42	42	39

	E-AA				
	N Scans				
Sample #	**1**	**2**	**3**	**4**	**5**
1	31	33	34	32	38
2	27	27	31	34	33
3	32	35	35	36	38
4	35	36	37	37	36
5	33	33	33	30	37
6	39	33	31	38	41
7	31	35	37	38	37
8	32	31	30	34	29
9	35	28	26	24	35
10	31	33	34	39	38

	D Scans				
Sample #	**1**	**2**	**3**	**4**	**5**
1	32	26	25	30	33
2	37	38	37	43	41
3	31	35	33	35	39
4	33	24	28	27	24
5	27	28	29	27	27
6	32	32	30	28	28
7	37	35	33	32	34
8	38	30	32	30	27
9	37	41	40	44	42
10	32	35	38	38	37

DW = Distilled Water
AA = Acetic Acid
V = Varnish
E = Enamel/No Varnish
N = Near
D = Distant

APPENDIX D: Canary Scans at T2

	V-DW				
	N Scans				
Sample #	**1**	**2**	**3**	**4**	**5**
1	26	22	20	17	20
2	26	26	20	20	21
3	20	18	18	20	15
4	21	20	19	19	16
5	17	18	19	21	20
6	16	19	20	23	20
7	24	25	22	22	28
8	24	24	23	19	17
9	20	20	19	19	25
10	14	15	20	18	20

	D Scans				
Sample #	**1**	**2**	**3**	**4**	**5**
1	17	18	15	13	17
2	22	19	25	28	28
3	20	18	15	15	20
4	24	15	19	22	16
5	22	16	17	16	19
6	14	14	15	19	19
7	19	13	15	15	19
8	11	19	16	14	20
9	15	18	19	17	19
10	20	24	19	19	20

	E-DW				
	N Scans				
Sample #	**1**	**2**	**3**	**4**	**5**
1	16	17	20	17	16
2	22	19	25	28	28
3	22	20	19	25	22
4	21	20	16	20	25
5	21	23	28	28	26
6	12	13	14	15	19
7	13	19	19	18	17

8	14	15	14	18	21
9	20	25	22	23	27
10	19	18	15	18	17

	D Scans				
Sample #	**1**	**2**	**3**	**4**	**5**
1	16	15	19	14	17
2	20	23	16	19	27
3	23	19	16	19	20
4	14	12	19	16	18
5	17	18	18	19	24
6	15	14	14	20	19
7	12	14	18	20	25
8	16	19	17	18	15
9	15	16	16	19	15
10	13	19	20	18	19

	V-AA				
	N Scans				
Sample #	**1**	**2**	**3**	**4**	**5**
1	47	53	53	52	53
2	38	40	44	38	42
3	34	37	34	37	40
4	45	42	40	43	33
5	28	34	35	34	36
6	37	43	39	40	39
7	37	40	41	34	36
8	39	38	38	39	45
9	30	34	31	31	32
10	39	39	39	40	46

	D Scans				
Sample #	**1**	**2**	**3**	**4**	**5**
1	45	45	45	45	52
2	22	20	19	19	18
3	27	35	33	33	34
4	24	24	18	17	18
5	34	34	38	24	31
6	41	42	44	43	46

7	40	44	41	39	34
8	30	36	37	37	37
9	34	30	31	33	30
10	45	43	45	45	46

	E-AA				
	N Scans				
Sample #	**1**	**2**	**3**	**4**	**5**
1	44	45	42	40	39
2	43	44	44	42	42
3	41	41	42	43	40
4	43	42	43	45	51
5	54	52	57	47	55
6	43	40	32	33	34
7	49	43	45	46	43
8	33	37	35	38	32
9	50	52	51	51	51
10	44	42	44	44	46

	D Scans				
Sample #	**1**	**2**	**3**	**4**	**5**
1	45	45	47	45	44
2	46	43	50	47	48
3	46	46	45	46	43
4	40	42	43	44	47
5	46	47	50	50	51
6	49	46	46	46	44
7	42	45	43	45	44
8	45	41	41	40	44
9	32	41	43	39	38
10	45	39	39	46	43

DW = Distilled Water
AA = Acetic Acid
V = Varnish
E = Enamel/No Varnish
N = Near
D = Distant

APPENDIX E: Canary Scans at T3

	V-DW				
	N Scans				
Sample #	**1**	**2**	**3**	**4**	**5**
1	21	19	18	18	19
2	23	21	20	17	19
3	20	27	26	23	24
4	17	19	21	18	23
5	23	20	19	16	19
6	14	17	23	20	15
7	18	20	17	19	13
8	15	16	24	19	26
9	18	25	17	18	22
10	19	20	17	25	25

	D Scans				
Sample #	**1**	**2**	**3**	**4**	**5**
1	21	17	18	25	16
2	19	20	18	19	19
3	18	20	19	21	25
4	15	15	17	19	19
5	14	15	9	13	18
6	20	19	20	19	22
7	16	15	18	15	18
8	14	10	15	14	19
9	19	19	19	20	19
10	17	18	19	17	19

	E-DW				
	N Scans				
Sample #	**1**	**2**	**3**	**4**	**5**
1	14	13	19	14	17
2	16	13	15	10	12
3	22	22	15	19	20
4	18	18	16	13	15
5	19	20	25	24	17
6	19	13	13	19	18
7	17	16	17	19	19

8	17	18	19	19	19
9	19	25	20	23	18
10	23	17	20	18	25

	D Scans				
Sample #	**1**	**2**	**3**	**4**	**5**
1	20	13	15	16	20
2	19	16	17	17	15
3	20	20	18	19	12
4	22	19	17	18	19
5	20	20	15	23	16
6	20	24	19	17	18
7	25	20	21	19	20
8	15	16	18	19	20
9	19	16	20	20	27
10	18	19	21	19	25

	V-AA				
	N Scans				
Sample #	**1**	**2**	**3**	**4**	**5**
1	42	42	47	49	50
2	43	41	41	48	50
3	45	43	43	36	39
4	25	31	27	21	20
5	34	33	37	34	38
6	41	35	43	44	40
7	27	35	35	36	36
8	53	51	51	50	45
9	36	34	31	34	31
10	31	33	36	39	30

	D Scans				
Sample #	**1**	**2**	**3**	**4**	**5**
1	46	47	47	49	49
2	40	44	47	49	45
3	34	40	32	33	30
4	32	26	39	37	31
5	35	39	37	39	41
6	47	45	49	51	50

7	32	34	34	37	40
8	38	39	40	44	37
9	34	33	34	30	33
10	39	45	46	45	46

	E-AA				
	N Scans				
Sample #	**1**	**2**	**3**	**4**	**5**
1	58	57	60	53	52
2	63	64	67	64	63
3	67	75	75	75	66
4	68	65	65	58	55
5	66	68	69	75	68
6	65	57	65	65	64
7	53	57	59	60	61
8	56	61	67	53	51
9	65	67	67	75	75
10	49	53	53	49	45

	D Scans				
Sample #	**1**	**2**	**3**	**4**	**5**
1	56	55	64	66	56
2	67	63	56	55	57
3	58	55	56	65	65
4	64	55	56	55	56
5	63	63	66	75	75
6	59	68	60	64	63
7	75	67	67	67	75
8	58	54	60	62	63
9	53	53	50	47	43
10	66	61	62	57	55

DW = Distilled Water
AA = Acetic Acid
V = Varnish
E = Enamel/No Varnish
N = Near
D = Distant

APPENDIX F: Repeat Canary Scans of T3 Samples

	V-DW				
	N Scans				
Sample #	**1**	**2**	**3**	**4**	**5**
1	19	20	19	19	18
5	18	19	23	17	20
10	18	17	19	24	26

	D Scans				
Sample #	**1**	**2**	**3**	**4**	**5**
1	22	19	18	16	17
5	18	12	16	15	15
10	19	16	18	20	13

	E-DW				
	N Scans				
Sample #	**1**	**2**	**3**	**4**	**5**
2	17	14	13	12	10
3	20	20	15	19	18
6	19	18	16	17	19

	D Scans				
Sample #	**1**	**2**	**3**	**4**	**5**
2	19	18	19	19	17
3	20	18	16	20	19
6	18	18	19	20	15

	V-AA				
	N Scans				
Sample #	**1**	**2**	**3**	**4**	**5**
4	28	23	27	27	26
7	25	29	30	31	37
9	37	38	39	34	29

	D Scans				
Sample #	**1**	**2**	**3**	**4**	**5**
4	33	31	26	35	39
7	33	38	35	39	37

9	35	33	29	30	30

	E-AA				
	N Scans				
Sample #	**1**	**2**	**3**	**4**	**5**
1	60	53	56	56	57
7	58	53	56	53	56
9	63	68	67	65	75

	D Scans				
Sample #	**1**	**2**	**3**	**4**	**5**
1	57	66	63	56	65
7	63	62	65	75	62
9	45	51	46	42	53

DW = Distilled Water
AA = Acetic Acid
V = Varnish
E = Enamel/No Varnish
N = Near
D = Distant